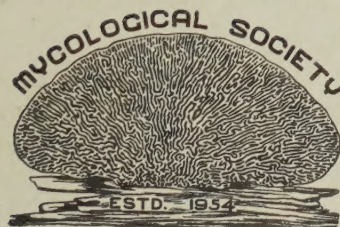


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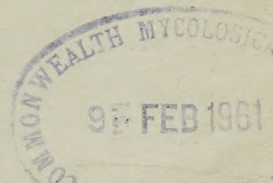
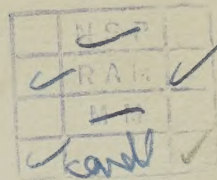
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INDIAN JOURNAL OF MYCOLOGICAL RESEARCH

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CONTENTS

Nos. 1 & 2, 1957

	PAGE
STUDIES ON DECAYS OF FELLED TIMBER—II. A ROT OF SĀL CAUSED BY <i>DAEDABA FLAVIDA</i> LÉV. SACHINDRANATH BANERJEE AND KANAK RANJAN SAMADDER	1-23
STRAINS OF <i>COLLETOTRICHUM FALCATUM</i> WENT IN WEST BENGAL. S. B. CHATTOPADHYAY AND ARATI SARKAR	24-28
CONTRIBUTIONS TO THE CYTOLOGY OF HYMENOMYCETES: IV. CYTOLOGICAL STUDIES IN <i>LENTINUS SUBNUDUS</i> BERK. AND <i>LENTINUS PRAERIGIDUS</i> BERK. SACHINDRANATH BANERJEE AND SUJATA SANYAL	29-35
SEXUALITY OF <i>TRAMETES CINGULATA</i> BERK. PRITINDRA MOHAN NAHA	36-39
STUDIES ON <i>IRPEX FLAVUS</i> KL.—I. ANTAGONISM BETWEEN DIFFERENT STRAINS AND THEIR HOST-RELATIONS. SACHINDRANATH BANERJEE AND RABINDRA PRASAD PURKAYASTHA	40-50
<i>CONIOPHORA CEREBELLA</i> PERS. A DRY ROT FUNGUS ON BUILDING TIMBER IN INDIA. B. K. BAKSHI, BALWANT SINGH AND P. S. REHILL	51-53

	PAGE
SPORULATION AND THE MECHANISM OF SPORE-DIS- CHARGE IN <i>TRAMETES LACTINEA</i> BERK.	
SACHINDRANATH BANERJEE AND NARAYAN CHANDRA BANERJEE	54-56
A PRELIMINARY NOTE ON VITAMIN DEFICIENCY IN <i>POLYSTICTUS SANGUINEUS</i> (L.) MEY.	
ASOKE KUMAR SINHA	57-58
EFFECT OF GROWTH SUBSTANCES ON <i>MERULIUS</i> <i>SIMILIS</i> B. & BR. IN CULTURE.	
SACHINDRANATH BANERJEE AND SANKAR MUKHOPADHYAY	59-63
FURTHER STUDIES ON USTILAGINALES : MORPHOLOGY AND SPORE-GERMINATION OF <i>PERICLADIUM</i> <i>GREWIAE</i> .	
N. C. JOSHI	64-65
ON THE PERFECT STAGE OF <i>CHAETOMELLA</i> EKL.	
N. MUKHERJEE	66-67

STUDIES ON DECAYS OF FELLED TIMBER—II. A ROT OF SĀL CAUSED BY *DAEDALEA FLAVIDA* LÉV.

By

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SAMADDER

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(With PLATES I to VI and 1 TEXT-FIGURE)

This investigation has been undertaken to study decay in stacked timber of Sāl (*Shorea robusta* Gaertn.f.) due to *Daedalea flavida* Lév.

The external morphology of the fungus growing on sāl logs has been fully described.

The mycelium from the infected logs has been isolated, grown in culture and compared with the original cultures of *D. flavida*, and found to be identical in every respect.

The gross characteristics of the rot have been described. The decay is mainly confined to the sapwood and with no apparent effect on the heartwood. The decayed sapwood shows characteristics of the 'stringy rot'.

Microscopic examination of the decayed wood shows that in an advanced stage of decay all the wood elements are attacked by thin-walled, hyaline hyphae with frequent clamp-connexions. The vessels are severely affected and often clogged by a mass of interwoven mycelial web within their lumina. The gummy substances of the medullary rays are gradually dissolved and eventually completely digested. Wood parenchyma also disintegrates and finally cracks appear within the wood.

Microchemical studies show the chemical changes in the wood during the process of decay. Both lignin and cellulosic materials are destroyed. Primarily *D. flavida* is a lignin-destroyer, but in later stages of decay it also consumes the cellulosic materials.

Decay resistance tests in the laboratory show that if the moisture-content of the host-wood is maintained above the 'fibre-saturation-point', *D. flavida* can cause considerable damage to both sapwood and heartwood. The sapwood is 'perishable' and the heartwood is 'non-resistant' to the attack of the fungus. Furthermore, the tests also indicate that the secondary mycelium has greater decaying capacity than the primary one.

Toxicity tests following wood-block method have been performed with zinc chloride, ascu 'A' and borax. Observations indicate that secondary mycelium has the least tolerance for toxic substances, and ascu 'A' is the best preservative in comparison with the other two.

Primary mycelium has been obtained from a single spore and by subsequent pairing experiments with several primary mycelia, secondary mycelium is produced.

Basidiospores from fresh fructifications have been found to germinate on various media of which the media containing malt-extract have been found to be best suited for profuse germination within a comparatively short time.

D. flavida, being a 'white-rot' fungus, gives 'strong' oxidase reactions when grown on malt-agar containing 0.5% gallic or tannic acid.

Cultural characteristics of the isolate and secondary mycelium on 2.5% malt-agar under ordinary conditions of light and temperature of the laboratory have been described.

The secondary mycelium and the isolates from the decayed wood have been found to fructify in wood-block cultures under controlled conditions in the laboratory.

INTRODUCTION

Sāl (*Shorea robusta* Gaertn.f.) is one of the important timber-producing hardwood species in India, and forms an integral factor in the national economy. Recently, demand for sāl logs has greatly been increased due to various developmental schemes in this country. As a result, problems inherent to the utilization of sāl wood have become evident. One of the difficult problems arises due to decay of logs, posts, lumbers, etc., by the wood-rotting fungi. In conformity with the general neglect of Forestry and Timber Pathology in this country, little attention was paid in the past to the decays of felled timbers. A series of investigations concerning the destruction and disintegration of stacked timbers caused by fungi have already been undertaken in this laboratory. The present paper forms a part of the series and reports on the decay of sāl caused by *Daedalea flavida* Lév.

Daedalea flavida Lév. is one of the common wood-rotting tropical polypores in India. In Bengal its fructifications are abundantly produced during the rainy season (July to October), but new fructifications can appear even up to December, provided the logs on which they grow get regular supply of water. The severity of damage of stacked logs of sāl due to the attack of the fungus can be witnessed in any timber-yard of Calcutta and its suburbs, especially during the wet months of the year. It has been estimated that about 20–30% of the logs are infected with the fungus and thus become non-merchantable imparting a heavy toll to the timbermen.

Daedalea flavida was first reported and described by Lévillé (1844)*. Like many other fungi it exhibits considerable variation in morphological characters, and, as such, the fungus appears in mycological literature under a variety of names. Berkeley described the species as *Daedalea hobsoni* (Saccardo, 1888). Lloyd (1911), while working on collections of the Polyporoid types at Leiden, mentions that the type collection of *Daedalea flavida* Lév. has been labelled as synonym of *Polystictus lenziteus* Lév., and according to him it is quite correct. He thinks that it is also synonymous with *Lenzites ochroleuca* Lév. and *Daedalea hobsoni* Berk. Bose (1920) at first described the fungus from Bengal as *D. hobsoni* Berk., but later publishes his researches (1938) on this species under the name *Daedalea flavida* Lév. He (1920-21) also thinks that fructifications of *Daedalea microzona* Lév., having regular pore-mouths and thinner context is a synonym of *Daedalea flavida* Lév., Presumably, *Trametes flavida* and *Lenzites flavida* (Lloyd, 1915) are also similar to the same fungus (Butler and Bisby, 1931). The host-range and geographical distribution of the fungus have recently been recorded by the writers in a previous communication (1956).

* Cited by Saccardo (1888).

SOURCE OF MATERIAL

The materials were collected during the rainy months of July to September, 1956, in the form of short, cylindrical sections of the infected logs of *Shorea robusta*, from the timber-yards of Calcutta and suburbs. The logs showing various stages of decay and with fructifications of *D. flavida* were selected and macroscopically examined in the field to note their characteristics as far as practicable. The cross-section-ends of the infected logs showed the presence of numerous, irregular rotted areas mainly in the sapwood. The demarcating line between the decayed sapwood and heartwood though being very sharp in most cases, no definite conclusion could be drawn in the field as regards the nature of the rot. For further detailed study, those decayed sections of the logs with fructifications were brought into the laboratory. Microscopic examinations of the sections from the rotted areas exhibited the presence of numerous, much-branched, septate hyphae with frequent clamp-connexions within the different wood-elements. The fruit-body of *D. flavida* has been found to be perennial and can resume growth under favourable conditions. In order to get regular supply of fresh fructifications, during the entire period of investigation, some of the logs with fructifications were kept moist in the laboratory. Moreover, the dried fructifications of the fungus when soaked with water readily revived and discharged viable spores copiously within a few hours.

THE BASIDIOCARP*

(PLATES I & II, FIGS. 1 to 4)

Fructifications sessile; usually dimidiate and attached to the substratum by its comparatively narrow base, sometimes somewhat reniform, and often encircling the substratum from which it grows, rarely more or less circular in outline and attached to the upper surface of the woody substratum by its central base, sometimes partly resupinate; applanate; solitary, rarely imbricate and laterally confluent; tough, coriaceous to corky, often woody on drying; dimension very variable, 12–22 cm. (30–45 cm.) across, 8–15 cm. deep and 1·5–2·5 cm. in thickness. Margin thin, usually entire sometimes broadly lobed, white in colour. *Upper surface* smooth, rugulose or uneven; marked with concentric, raised or depressed, narrow or broad zones; colour when fresh Cinnamon-Buff or Clay colour, sometimes Zinc-Orange or Cinnamon-Orange with a few narrow Cinnamon-Brown rings towards the periphery, zonations becoming Light-Buff, Warm-Buff or Ochraceous-Buff and occasionally with a few Tawny-Olive rings towards the margin on drying. *Context* coriaceous to corky; about 5–12 mm. in thickness; white when fresh becoming concolourous with the hymenium on drying. *Hymenial surface* smooth; Milk-White when fresh, but changing to Light-Buff, Light-Ochraceous-Buff or Ochraceous-Buff on drying; pore-mouths typically labyrinthiform, in part at least in some

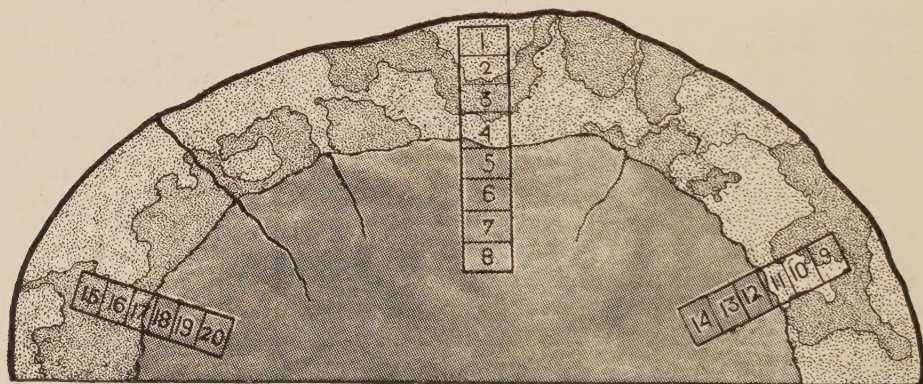
* Colour according to Ridgway (1912).

poroid at first, often becoming partly lamellate which anastomose with one another by thick dissepiments having obtuse edges, transitional stages from poroid to lamellate types quite common, sometimes torn into teeth; a narrow sterile zone at the margin always present; without any shining lustre; pore-tubes wide, deeping unequally into the substance of the context and not forming a distinct layer, about 8–15 mm. long. *Basidia* elongated and elevated; tetrasterigmatic and quadrisporous; dimensions about $12.5-20 \times 1.5-5.5 \mu$; with simple clamp-connexions at the base. *Basidiospores* hyaline, more or less oval but flattened at one side and with an excentric apiculus; thin-walled; smooth; dimensions $4-6 \times 1.5-2.5 \mu$.

DECAY OF SĀL

Isolation and identification of the causal organism of the rot

In order to study the causal organism in culture, the mycelia from the rotted tissues of the infected logs were isolated. Isolations were made from different portions of the rotted areas of the same and different logs which bore externally large number of fructifications of *D. flavida*. Following the method of Banerjee (1955), small pieces of infected wood were obtained from carefully selected regions of the logs and serially numbered (Text-fig. 1). After surface sterilization, small pieces of internal tissues from each block were aseptically transferred to sterile 2% malt-agar slants. The cultures were then kept under ordinary conditions of temperature (28–32°C.), humidity (62%–67%) and diffused light of the laboratory.



TEXT-FIG. 1. Part of a cross-section of a decayed log of *S. robusta* showing the areas from which isolations were made (Diagrammatic).

Within a week mycelia grew out from the inocula obtained from the areas 1, 2, 3, 4, 9, 10, 11, 15, 16 and 17 and spread over the surface of the slants, while, the inocula from areas 6, 7, 8, 13, 14, 19 and 20 failed to produce any mycelium. It can, therefore, be concluded that the central core of the heartwood is entirely free from fungal hyphae, and is definitely in a

healthy condition while, the areas 5, 12 and 18 lying in contact with the sapwood show the presence of mycelia in them, although they appear to be sound macroscopically.

From these isolates several subcultures were made in 2% malt-agar slants which later served as stock cultures for further study. When critically examined, all the isolates exhibited identical macroscopic and microscopic characteristics and resembled one another in essential details. These were then compared from all aspects with the polysporus mycelium of *D. flavida* already made available for the purpose and found to be identical with the latter. The identity was further confirmed later when the isolates produced typical fruit-bodies of *D. flavida* under cultural conditions (PLATE VI, FIG. 26).

Macroscopic characters of the rot

The first externally visible symptom in the field is the formation of typical brackets of *D. flavida* of different sizes on the surface of the logs (PLATE III, FIG. 5). The cross-section-ends of the logs, however, show considerable sign of decay in the wood, and this appears to be very distinctive in character. In the early stages the demarcating line between the sapwood and heartwood is more prominent than that in the normal wood and this seems to be due to some gross changes in the appearance of the sapwood. The rot at this stage is confined entirely to the sapwood, which forms a conspicuous decayed zone surrounding a central practically unaffected heartwood.

In the first stage of decay the sapwood becomes pale brown in colour, which appears to be lighter than that of normal sapwood. With the gradual advancement of decay, the colour turns to pale yellow and finally to yellowish white, and this shows that considerable bleaching of the sapwood has gradually taken place. At first, the rotted areas appear at the periphery as irregular pale brown coloured areas, which are often separated from one another by broad or narrow brownish black zones (PLATE III, FIGS. 6 & 7). Gradually, as the decay proceeds, these rotted areas extend inwards upto the heartwood and also spread longitudinally and laterally as evidenced in both transverse and longitudinal sections (PLATE III, FIGS. 6-8). Eventually the rots coalesce to form larger areas and the individuality of the blackish brown regions in the sapwood becomes partially lost. Numerous rot-pockets become conspicuous at this stage (PLATE III, FIG. 6). Macroscopically the heartwood appears to remain unaffected.

In the later stages of decay, the texture of the sapwood loses its toughness and becomes soft, light and somewhat spongy. The wood also appears to be highly bleached, compressible, full of numerous rot-pockets consisting of masses of yellowish white-coloured fibres and form a stringy rot (PLATE III, FIGS. 6 & 7). The pockets are large, hollow and pale yellow to yellowish white in colour. No fungal hyphae have been externally recognized in the pockets. The reddish-brown heartwood, on the other hand, remains apparently unaffected except for somewhat intensification of the colour in the regions directly abutting the decayed sapwood (PLATE III, FIG. 8). The fungus has been mentioned to cause a white spongy rot in

Shorea robusta by Bagchee, Puri and Bakshi (1955) and in other literature*, but the nature seems to be rather stringy than spongy. The stringy nature of the rot becomes obvious as the decayed portion of wood cannot be polished, as its fibres break off in minute needles over the sand paper owing to the 'brashy' nature of the decayed wood.

Microscopic details of the rot

Infected logs collected from the timber yards and bearing fructifications of the fungus were found to be unsuitable for obtaining satisfactory sections, although the internal mycelium was in good conditions for the study. This is due to the fact that the rotted samples of wood were already in an advanced stage of decay, and therefore, they had become rather 'brash'. As the actual age of the samples of wood collected from the timber-yards could not be determined, owing to the non-availability of necessary data, they were selected for studying the latter stages of decay only. The early stages of decay were studied by following the method proposed by Banerjee and Sinha (1954). Sound, rectangular sapwood blocks ($2'' \times 1'' \times \frac{1}{2}''$) were exposed aseptically to the attack of *D. flavida* growing on 2% malt agar slants in Kolle flasks, as has been described in details in a later chapter on 'decay-resistance tests', and kept under controlled conditions for a period of four months. Four sets of test-samples infected in this way were taken out in batches, one set at the end of each month in order to obtain representative materials for observing gradual process of attack and consequent progress of decay in early stages. These test-samples, as well as samples from decayed wood obtained from nature, were then fixed and preserved in Formal-Acetic-Alcohol (70% Alcohol 85 c.c., Gl. acetic acid 5 c.c., and Formalin 10 c.c.) for future use.

Before sectioning, the test-samples, in almost all cases, were softened by special treatment, as recommended and followed in the Forest Products Research Laboratory, Princes Risborough, England. The wood-pieces were kept immersed in a vessel of gently boiling water until they became completely water-logged and sank. This process was found to be quite satisfactory for the wood showing late stages of decay, but sectioning of wood with early stages of decay required further softening. This was done by transferring the water-logged wood blocks to a mixture of equal volumes of glycerine and methylated spirit, and left as such for 2 to 3 days before sectioning. Sound wood-samples were also treated in a similar manner, and these served as controls. Transverse, radial-longitudinal and tangential-longitudinal sections were cut both by free hand and with the aid of a microtome. Microtome sections about $15-20\mu$ thick were cut, but in general, free hand sections were found to be more suitable in rendering the internal mycelium visible without much distortion. Of the various differential staining methods, the combination stains Saffranin and Picro-Aniline blue, as suggested by Cartwright (1929) proved to be very suitable for detecting the hyphae in the woody tissues. In the resulting prepara-

* List of Common names of Indian Plant Diseases compiled by the Plant Pathology Committee of the Indian Council of Agricultural Research (1950).

tions the lignified cell-walls of the wood elements showed various shades of red, and the fungal hyphae appeared in deep blue colour.

In the early stages of decay fungal hyphae are usually confined to the vessels and wood parenchyma, but the distribution of the hyphae is not uniform throughout. A few hyphae, however, are noticeable in the medullary ray cells. The hyphae are thin-walled, hyaline, sparsely branched, distantly septate, and with frequent clamp-connexions, almost at each septum. These hyphae can be easily distinguished into two types. Hyphae of comparatively broader diameter (2.5–4.5 μ) are very frequent in the lumina of the vessels and wood parenchyma, and are deeply stainable. They pass longitudinally through the wood elements, and produce conspicuously narrow branches (1–1.5 μ in diameter). These branches are found predominantly in other wood elements, and penetrate the vertical walls of the elements in transverse direction. Both the types of hyphae are full of granular protoplasmic contents which take up the stain deeply. As the decay proceeds, the mycelium gradually ramifies, and in the lumina of the vessels loosely interwoven mycelial web is formed (PLATES IV, FIGS. 10–12). It is very probable that in the initial stages of attack the hyphae of broader diameter simply pass, without any resistance, in the longitudinal direction through the wide lumina of the vessel (PLATE IV, FIG. 11). Gradually, however, the wood parenchyma and medullary ray cells are invaded by their branches. The tracheids and wood fibres, except a few, are the least affected elements at these stages. Radial penetration and consequent invasion of the neighbouring elements by the narrow branches are mainly through the pits of the thick-walled vessels. In the later stages of decay, the pits of different wood elements become very much enlarged in their diameter due to dissolution of the walls by fungal action, so that the hyphae pass loosely through them. The hyphae may also penetrate the cell walls directly at right angles to them forming circular to oval bore-holes. The smooth and moulded contours and not ruptured or splintered walls of the bore holes and pits through which hyphae pass radially, support Proctor's (1941) theory of cell-wall penetration due to enzymic activity of the fungus.

As the decay advances, cell-walls of groups of parenchyma cells are rendered very thin and ultimately disintegrated, forming spongy areas between the wood fibres. As a consequence, the compactness of the wood is gradually lost, and in later stages of decay cracks appear in the wood, mostly between the groups of fibres, splitting them into several irregular strands (PLATE IV, FIGS. 10 & 18). Dark brown gummy materials of the medullary ray cells (PLATE IV, FIG. 13) are gradually removed and the cell-cavities become filled with masses of hyphae (PLATE IV, FIG. 12). Gradually these cells are completely bleached and are without gummy substances (PLATE IV, FIGS. 14 & 16). In still later stages of decay the walls become comparatively thin, and ultimately break down forming conspicuous gaps in between the fibres and occupying the positions of the medullary ray cells (PLATE IV, FIGS. 15 & 17). This is in harmony with the complete bleaching of the wood-blocks as has been observed during decay-resistance tests.

Microchemical studies

In order to gather preliminary informations about the chemical changes that take place with the progress of decay microchemical tests, that are generally employed, for lignin and cellulose in sound and partially decayed wood, had been performed. The usual practice of staining the sections of the wood with phloroglucin-HCl and chlorzinc-iodine to detect the presence of lignin and cellulose respectively had been employed. Phloroglucin-HCl turns lignified walls red, while chlor-zinc-iodine turns cellulosic walls blue. The results obtained by staining with phloroglucin-HCl were further verified by 'Maule treatment' in which the sections were treated with 1% aqueous potassium permanganate solution for 10-30 minutes washed in distilled water, placed in dilute HCl (sp.gr. 1.06) for about 3-4 minutes until the colour disappeared and washed again. On mounting the sections in ammonium hydroxide solution, lignified walls took red stain. The results indicated by chlor-zinc-iodine had also been verified by treating the sections with iodine-potassium-iodide and sulphuric acid (65%). As a corroborative evidence of the findings obtained by employing the above tests, the combination stain Gentian violet and Bismarck brown, differentiating lignified (violet staining) from non-lignified (brown staining) structures had been used. The results of these staining reactions are given in Tables 1 to 5.

Table 1. *Results of staining for lignin in normal and partially decayed wood of Shorea robusta with phloroglucin-HCl.*

Elements	Normal wood	Partially decayed wood
Vessels	Primary walls deep red; secondary walls light red. Tyloses almost colourless.	Primary walls light red; secondary walls pink to almost colourless towards the lumen. Fragments of heavily-decayed vessels almost colourless.
Wood parenchyma	Primary walls red; secondary walls light red.	Primary walls faintly pinkish; secondary walls pale pink to colourless.
Tracheids	Primary walls deep red; secondary walls light red to pinkish red.	Primary walls light-red; secondary walls pink to almost colourless.
Wood fibres	Primary walls deep red; secondary walls faintly red or pink.	Primary walls pink; secondary walls pale pink in patches to colourless.
Ray cells	Primary walls light red; secondary walls pinkish.	Primary walls light red; secondary walls almost colourless.

Table 2. *Results of staining for lignin in normal and partially decayed wood of Shorea robusta with 'Maule treatment' (KMnO₄-HCl-NH₄OH)*

Elements	Normal wood	Partially decayed wood
Vessels	Primary walls red; secondary walls pinkish to almost colourless.	Primary walls pale pink; secondary walls colourless.
Wood parenchyma	Primary walls light red; secondary walls pale pink.	Primary walls pale pink; secondary walls pale pink in patches to colourless.
Tracheids	Primary walls red; secondary walls somewhat pinkish towards the lumen.	Primary walls pale pinkish red; secondary walls colourless towards the lumen.
Wood fibres	Primary walls bright red; secondary walls paler red and pinkish towards the lumen.	Primary walls dirty red; secondary walls pinkish red to almost colourless towards the lumen.
Ray cells	Pale orange red in patches.	Pale pinkish to orange red in patches.

Table 3. *Results of staining for cellulosic materials in normal and partially decayed wood of Shorea robusta with chlor-zinc-iodine.*

Elements	Normal wood	Partially decayed wood
Vessels	Secondary walls pale blue in patches.	Secondary walls almost colourless.
Wood parenchyma	Secondary walls pale blue.	Secondary walls faint blue to colourless.
Tracheids	Some secondary walls faintly blue.	Secondary walls bluish to almost colourless.
Wood fibres	Secondary walls light blue to colourless.	Secondary walls almost colourless; in some cases faintly blue.
Ray cells	Light bluish green	Light bluish green.

Table 4. *Results of staining for cellulosic materials in normal and partially decayed wood of Shorea robusta with iodine-potassium-iodide and sulphuric acid.*

Elements	Normal wood	Partially decayed wood
Vessels	Primary walls golden brown; secondary walls faintly greenish blue.	Primary walls yellowish; secondary walls yellowish green.
Wood parenchyma	Primary walls deep yellowish brown; secondary walls faintly blue.	Primary walls yellowish; secondary walls faintly blue.
Tracheids	Primary walls pale brown; secondary walls mostly faint blue.	Primary walls greyish yellow; secondary walls pale greenish yellow in patches.
Wood fibres	Primary walls deep brown; secondary walls blue.	Primary walls greyish brown; secondary walls greenish yellow to faint blue.
Ray cells	Primary walls yellow; secondary walls pale bluish green.	Primary walls yellowish; secondary walls faint bluish green.

Table 5. *Results of staining for lignin and cellulosic materials in normal and partially decayed wood of Shorea robusta with gentian violet and bismarck brown*

Elements	Normal wood	Partially decayed wood
Vessels	Primary walls deep violet; secondary walls light violet. Tyloses dirty brown.	Primary walls light violet; secondary walls yellowish brown to colourless. Tyloses dirty brown.
Wood parenchyma	Primary walls deep violet; secondary walls light violet to brownish yellow.	Primary walls light violet; secondary walls greyish brown to colourless.
Tracheids	Primary walls violet; secondary walls light violet but brownish around the lumen.	Primary walls light violet; secondary walls yellowish brown in patches.
Wood fibres	Primary walls violet; secondary walls in golden brown patches near the lumen; violetish near the middle lamella.	Primary walls light violet; secondary walls pale violet in patches to yellowish brown.
Ray cells	Primary walls violet; secondary walls light violet in patches; mostly yellowish brown.	Primary walls lightviolet; secondary walls greyish brown.

From Tables 1 and 2 the presence of lignin in both primary and secondary walls of the normal wood elements is evident. But the relative concentration in the primary walls is greater than that in the secondary walls. In partially decayed wood, on the other hand, the intensity of staining reactions of both primary and secondary walls of all the wood elements is variable, and indicates a gradual delignification of the lignified parts. The pink, pale pink to almost colourless conditions of the secondary walls of all the wood elements indicate delignification to a remarkable extent. There is also considerable delignification in the primary walls of all the wood elements excepting in the ray cells as indicated by their lighter colour reactions than that in the normal wood. It is clearly evident that the secondary walls suffered greater delignification than the primary walls.

From Tables 3 and 4 it is revealed that the cellulosic materials have also undergone digestion during the process of decay, but the destruction is much less than that suffered by the lignified structures. It is also clear that cellulose is consumed from the secondary walls of all the wood elements, such as vessels, wood parenchyma, tracheids and wood fibres. Walls of ray parenchyma remain bluish green as in the normal wood, and this indicates that no appreciable loss of cellulosic materials has taken place at least, in the early stages of decay. Staining with Gentian violet and Bismarck brown also supports these findings. It is also revealed by these staining reactions that the decay of wood is due mainly to loss of lignin present in the primary and secondary walls. It appears that digestion of cellulosic materials also goes on simultaneously but at a much slower rate in the early stages of decay, when the wood can withstand sectioning. No further comment can be made as to whether the fungus in its later

stages of decay can consume cellulosic materials at a much higher rate than that in the earlier stages, since the wood at these stages is useless for sectioning, as it has become 'brash'.

Sulphuric acid test.—Following Ritter (1925), sections of natural and partially decayed wood, corresponding to those used for microchemical tests were treated with 72% sulphuric acid on slides. The cells of the sections of normal wood swell immediately after the acid treatment, break apart and dissolution of the secondary walls occurs, leaving on the slides a mixture of floating lamellae, vessels, ray cells and wood parenchyma. Sections of the partially decayed wood, on the other hand, when treated similarly give the same reaction as in the case of normal, but there the action of sulphuric acid is much less vigorous and less rapid. The violent action in the case of normal wood is due to the presence of large quantities of cellulosic substances in it which in partially decayed wood are present in a somewhat lower concentration, as indicated by less vigorous action. Though this test is not conclusive in itself, it may be concluded at least, that cellulosic materials are present in the partially decayed wood in comparatively lower concentrations than those in the sound wood.

All the above microchemical staining reactions indicate that *Daedalea flavida* can also consume some cellulose in addition to its great preference for lignin destruction in the early stages of decay. The most extreme decay will probably show the digestion of cellulose in comparatively much greater proportion.

Decay resistance

In the past the natural resistance of various timbers to the attack of wood-rotting fungi has been tested in the laboratory, and various methods of decay resistance were suggested by a number of investigators.

In all cases the fundamental principle underlying the technique is to expose small samples of sterilized test-pieces of sound wood to the attack of pure cultures of wood-rotting fungi under controlled conditions. The amount of decay resulting from fungal attack is calculated in terms of final loss in dry weight of the test-pieces. For the present investigation the writer had mainly followed the method suggested by Banerjee (1955) with some modifications as follows :—

Small rectangular, sound sapwood and heartwood blocks ($2'' \times 1'' \times \frac{1}{2}''$) were cut from a *sāl* log (*S. robusta*) of moderate age (12" in diameter) and planed. These test-blocks were then serially numbered, and were dried in an oven at 60°C. to a constant weight. They were then soaked in distilled water under reduced atmospheric pressure till the average moisture-content of the individual wood-block rose much above the 'fibre-saturation-point'. Eight such blocks were put on a moist cotton pad in a Kolle flask, plugged, and sterilized in an autoclave for 10 minutes at 15 lbs. pressure.

The sterilized wood-blocks were then exposed to primary and secondary mycelia of the test fungus already grown on sterile 2% *malt-agar* slants in Kolle flasks. These flasks, each containing four samples of wood, were

kept under ordinary conditions of temperature (28–32°C.) and diffused light of the laboratory.

In order to determine the average moisture content of the wood-blocks just before their exposure to fungal action, another set of test-pieces of both sapwood and heartwood, soaked and sterilized in the same way, were carefully weighed. The initial moisture-contents of the test-pieces were expressed in percentage and calculated by following the relationship* pointed out by Savory (1954).

Following the recommendation of Cartwright and Findlay (1946), a period of four months' exposure of the blocks to fungal attack, for obtaining significant results in decay-resistance tests, was employed. After the test period the blocks were taken out, and after careful scraping of the superficial mycelium, weighed in order to find out their moisture-content at this stage. The test-blocks were then examined to record external manifestation of decay and dried in an oven at 60°C. to a constant weight. The final dry weights of test-pieces when subtracted from their original dry weights gave the loss in weight due to fungal attack. The results thus obtained are given in Table 6.

Table 6. *t*-test of comparison of loss in dry weight (%) of sapwood and heartwood of *Shorea robusta* exposed to primary and secondary mycelia of *Daedalea flavida*

Type of wood	Kind of mycelia	Number of replicates	Range	Mean	S.E.	<i>t</i>
Sapwood	Primary	12	23.50–35.56	29.34	±1.47	4.8
	Secondary	12	29.98–50.06	40.41	±1.76	
Heartwood	Primary	12	2.03–11.34	6.34	±1.01	6.1
	Secondary	12	9.66–17.95	13.93	±0.73	

Table 6 clearly indicates that the loss in weight of heartwood is less than that in the sapwood on the exposure to either primary or secondary mycelium. The mean loss in case of sapwood during the test period is 29.3 ± 1.47 in case of primary mycelium, and 40.41 ± 1.76 in case of secondary mycelium; the same in heartwood are 6.33 ± 1.01 and 13.93 ± 0.73 respectively. Further in the comparison of means of primary and secondary for sapwood, the value of $t = 4.8$ for 22 degrees of freedom is highly significant showing that there is a real difference in the activity of two types of mycelia. A similar significant difference is also clear from the $t = 6.1$ for heartwood under similar conditions.

* Moisture Content = $\frac{\text{Wt. of water present in the wood} \times 100}{\text{Oven dry wt. of the wood}}$

Following Findlay's (1946) classification, the sapwood of *S. robusta* exposed to secondary mycelium may be classed as 'perishable', and the same when exposed to primary mycelium is 'non-resistant'. On the other hand, the heartwood blocks exposed to primary and secondary mycelia fall under 'moderately resistant' and 'non-resistant' groups respectively. The controls in all the cases, which were free from fungal mycelium, show no appreciable loss in weight of the test-blocks. Bagchee, Puri and Bakshi (1955), carrying out similar experiments, concluded that the fungus caused a 10% loss in weight of *sāl* sapwood in four months. As detailed informations regarding the conditions of their experiment such as, temperature, light, the size of the test-pieces and the types of mycelia they used are lacking, the writers will refrain from making any comment on this very wide variation in the two results.

The foregoing results clearly exhibit the fact that *D. flavida* attacks both sapwood and heartwood under controlled conditions in the laboratory, and there exists a definite relationship between the types of mycelia and the nature of the wood. The severity of decay is extreme in the sapwood, and in the heartwood region it is moderate.

The initial moisture-content of the sapwood and heartwood blocks before the experiments were on the average about 80% and 60% respectively, which can be regarded as optimum moisture-content as pointed out by Kaufert (1936). After the experimental period, the sapwood blocks attacked by the primary and secondary mycelia retained the average moisture-content of about 72% and 68% respectively, while that of heartwood blocks under identical conditions were 49% and 46% respectively. As no control measure had been adopted to maintain the exact moisture-content of the wood blocks, they were not constant during the experimental period as evidenced from above. But it is clear that the normal activity of the fungus did not suffer in any way, as the moisture-contents are always found to be much above the 'fibre-saturation point'.

The test-blocks at the end of the experimental period show macroscopic manifestations of decay. The sapwood blocks become light, soft, and porous. Areas which have undergone severe damage, have rough and loose fibrous texture, and crumble into 'brashy' needles of woodelements, when a rubbing pressure is applied. That the sapwood has become 'brash' is evident when tested with a knife. The fibres break short over the top of the knife owing to their loss of toughness. The sapwood blocks exposed to secondary mycelium, in most cases, have undergone complete bleaching covering the entire wood-block and all its surfaces (PLATE III, FIG. 9b), while those exposed to primary mycelium show variable extents of bleached areas indicating that the bleaching is but partial (PLATE III, FIG. c-g). The bleached areas have no sharp outline and are not streaked, spotted or banded by darker and lighter colours.

The heartwood blocks show similar external manifestations of decay, but the bleaching is not so prominent as in the sapwood. As a result of bleaching, the wood blocks become yellowish white in colour, while the normal colour of sapwood and heartwood is pale brown and brown to reddish-brown respectively.

Toxicity test

Though hundreds of chemicals and various combination mixtures of different chemicals have been suggested as wood preservatives, the ideal preservative, according to Cartwright and Findlay (1946), should possess the most important character that it should have a high toxicity towards wood-rotting organisms. Of the various preservatives, three different water soluble preservatives, viz., Zinc chloride, Ascu 'A' and Borax were selected. All the three preservatives possess the characteristics, as stated by Cartwright and Findlay, of ideal preservatives.

Weighed wood blocks soaked and impregnated with a graded series of concentrations of the three preservatives were subjected to the attack of both primary and secondary mycelia grown on 2.5% *malt-agar*. Since the sapwood of *S. robusta* is 'non-resistant' and 'perishable' to the attack of *D. flavida*, it had been selected for the preparation of test-pieces of toxicity test. The blocks (5 cm. $\times$ 2.5 cm. $\times$ 1.5 cm.) were dried and sterilized by heating in a drying oven at 100°C. for about 18 hours. The initial oven-dry weights were recorded. The concentrations used were different for different preservatives and in each case were determined beforehand by growing the two types of mycelia on 2% *malt-agar* containing different concentrations of the preservative. The blocks were impregnated with the preservatives by immersing them in the solution at laboratory temperature (23°C.) and then exposed to reduced atmospheric pressure, by connecting the vessels containing the solution to a suction pump. When the blocks sank down the pressure was released, and they were allowed to remain submerged in the solution for about 2 hours, after which they were completely saturated. After wiping off the superficial liquid they were weighed again. The amount of preservative absorbed was then calculated on the basis of the solvent absorbed, and the concentration of the solution expressed in percentage. The actual quantity of the preservative present in the wood was then calculated as kilograms per cubic metre of wood. At each concentration four blocks were treated and the results were averaged on these four blocks. A period of four months' test in ordinary temperature, humidity and diffused light of the laboratory had been employed in order to obtain significant results. After four months the final oven dry weights of the test-blocks and the usual manifestation of decay, if any, were recorded. The final dry weights of the test-pieces when subtracted from their original dry weights, gave the loss in weight of the wood-blocks due to fungal action for four months. The results are given in the Tables 7—12.

It is to be noted from Tables 7 to 12 that of the three preservatives, ascu 'A' is the most powerful, since both the types of mycelia have least tolerance for it. The sapwood treated with ascu 'A' is resistant' to the attack of both primary (PLATE V, FIG. 25) and secondary (PLATE V, FIG. 24) mycelia, when approximately 0.40–0.45 kilogram of the preservative is present per cubic metre of the wood. The 'very resistant' nature of the sapwood exposed to primary mycelium is probably due to the presence of increased quantity (0.55 kilogram/metre cube approx.) of the preservative together with the less vigorous activity of the primary mycelium, as already indicated in decay-resistant tests. Zinc chloride is less effective

Conc. of treating solution	Serial No.	Initial dry wt.(gms.)	Wt. after treatment (gms.)	Absorption Kg/m ³	Final dry wt.(gms.)	Loss in dry wt.(gms.)	Loss%	Condition of samples after test
0.05%	1.	12.195	20.235	0.22	10.250	1.94	15.91	Partially decayed
	2.	11.855	20.095	0.22	9.585	2.17	19.99	
	3.	12.475	20.755	0.21	10.560	2.9	15.24	
	4.	12.035	20.055	0.21	9.820	2.21	18.37	
	Average loss in dry weight = 17.38%							
0.1%	1.	11.775	18.175	0.34	10.725	1.05	8.92	Partially decayed
	2.	12.985	18.455	0.33	11.015	1.27	9.76	
	3.	12.445	21.855	0.50	11.195	1.25	10.04	
	4.	11.275	20.445	0.49	10.200	1.07	9.49	
	Average loss in dry weight = 9.55%							
0.15%	1.	11.815	19.055	0.52	11.115	0.7	5.93	Partially decayed
	2.	11.835	20.550	0.52	11.350	0.58	4.90	
	3.	11.930	20.450	0.69	11.375	0.56	4.69	
	4.	11.640	20.175	0.69	11.015	0.65	5.68	
	Average loss in dry weight = 5.30%							

Conc. of treating solution	Serial No.	Initial dry wt.(gms.)	Wt. after treatment (gms.)	Absorption Kg/in ³	Final dry wt. (gms.)	Loss in dry wt. (gms.)	Loss%	Condition of samples after test
0.01%	1.	11.245	19.145	0.04	10.105	1.14	10.14	Partially decayed
	2.	12.425	19.600	0.03	11.375	1.05	5.45	
	3.	11.435	19.125	0.04	10.050	1.38	12.11	
	4.	11.175	18.565	0.03	10.100	1.07	9.62	
Average loss in dry weight = 10.08%								
0.05%	1.	11.095	19.030	0.21	10.145	0.95	8.56	Partially decayed
	2.	12.325	19.965	0.20	11.520	0.80	6.53	
	3.	11.055	17.685	0.17	10.300	0.75	6.83	
	4.	12.235	21.355	0.24	11.925	0.31	2.53	
Average loss in dry weight = 6.11%								
0.1%	1.	11.355	18.310	0.37	11.025	0.33	2.91	Almost sound
	2.	12.295	20.550	0.44	11.963	0.33	2.68	
	3.	12.850	21.355	0.45	12.605	0.25	1.95	
	4.	10.955	18.905	0.42	10.680	0.28	2.55	
Average loss in dry weight = 2.52%								

Table 11. *Toxicity test with sapwood blocks of Shorea robusta treated with borax and exposed to primary mycelium of Daedalea flavida for a period of 4 months at 28–32°C.*

Conc. of treating solution	Serial No.	Initial dry wt. (gms.)	Wt. after treatment (gms.)	Absorption Kg/m³	Final dry wt. (gms.)	Loss in dry wt. (gms.)	Loss%	Condition of samples after test
0.1%	1.	12.320	19.575	0.37	10.355	1.96	15.90	Partially decayed
	2.	12.650	21.370	0.46	10.725	1.93	15.25	
	3.	11.695	20.070	0.45	9.810	1.89	16.16	
	4.	11.025	18.925	0.42	9.195	1.83	16.59	
Average loss in dry weight = 15.75%								
0.15%	1.	11.760	19.725	0.63	11.105	0.65	5.53	Partially decayed
	2.	12.500	19.525	0.64	11.950	0.55	4.40	
	3.	11.750	20.750	0.72	11.290	0.46	3.91	
	4.	10.620	17.370	0.54	9.985	0.64	6.03	
Average loss in dry weight = 4.96%								
0.2%	1.	11.165	18.820	0.81	10.755	0.41	3.67	Partially decayed
	2.	11.690	20.050	0.89	10.985	0.70	5.98	
	3.	13.025	20.725	0.82	12.175	0.85	6.52	
	4.	11.705	20.150	0.89	11.055	0.65	5.55	
Average loss in dry weight = 5.43%								

Table 12. *Toxicity test with sapwood blocks of Shorea robusta treated with borax and exposed to secondary mycelium of Daedalea flavida for a period of 4 months at 28–32°C.*

Conc. of treating solution	Serial No.	Initial dry wt. (gms.)	Wt. after treatment (gms.)	Absorption Kg/m ³	Final dry wt. (gms.)	Loss in dry wt. (gms.)	Loss%	Condition of samples after test
0.05%	1.	12.745	22.455	0.26	10.150	2.59	20.32	Partially decayed
	2.	12.675	21.940	0.24	10.305	2.37	18.69	
	3.	11.625	19.615	0.21	10.020	1.61	13.79	
	4.	12.575	20.350	0.19	10.075	2.50	20.00	
Average loss in dry weight = 18.20%								
0.1%	1.	10.395	17.365	0.37	9.010	1.38	13.59	Partially decayed
	2.	12.155	20.950	0.46	10.875	1.28	10.65	
	3.	11.455	19.670	0.43	9.185	2.27	19.13	
	4.	12.350	19.420	0.39	10.435	1.91	15.32	
Average loss in dry weight = 14.67%								
0.15%	1.	12.300	20.650	0.65	11.725	0.57	4.63	Partially decayed
	2.	11.955	19.775	0.63	11.205	0.75	6.30	
	3.	12.595	19.785	0.59	11.785	0.81	6.42	
	4.	12.175	20.870	0.68	11.505	0.67	5.49	
Average loss in dry weight = 5.71%								

than ascu 'A' since for each cubic metre, approximately 0.60–0.70 kilograms of the preservative are required, to make the wood 'resistant' to the attack of the fungus. Borax appears to be the least effective, and a quantity as high as 0.60–0.70 kilograms per cubic metre is to be applied, provided which the wood is 'moderately-resistant'.

CAUSAL ORGANISM

Material

The causal organism has been studied in culture. Three types of mycelia were employed, *viz.*, (i) primary mycelium without clamp-connexions, (ii) secondary mycelium with clamp-connexions, and (iii) isolates obtained from rotted wood. Several single-spore-cultures were made following the usual dilution method in which a spore suspension in sterile distilled water was spread on 1% *dextrose-agar* plate, and the superfluous water was carefully drained off. After 12 hours the plates were examined under the microscope, and well, germinated and isolated spores were selected, punched out by means of a sterile cutter and transferred to 2% *malt-agar* slants. In all cases mycelia developed within the tubes, and after careful examinations they were all found without clamp-connexions indicating the fact that all of them were in primary condition. Subsequent mating experiments with these primary mycelia, typical secondary mycelia with clamp-connexions were obtained. The method of isolation of the organism from rotted tissues had already been described.

Spore-germination

The spores were obtained from fresh sporophores collected in the field and also from those produced in wood-block cultures in the laboratory. They were sown aseptically in hanging drops of different media in sterile van Tieghem cells, and incubated in diffused light at 30°C. The media used were as follows : tapwater, distilled water, and distilled water containing traces of alcohol and lactic acid; 1% solutions of malt extract, sucrose, glucose and dextrose; 2% pure agar, 2% *dextrose-agar*, 2% *malt-agar* and *potato-dextrose-agar*. Alcohol and lactic acid were added to distilled water after sterilization. Spore-deposits on perfectly dry and sterilized slides within Petri-dishes were also kept under identical conditions. Observations were made at regular intervals of 3 hours in order to ascertain whether the spores had germinated or not. The results of these observations are given in Table 13. The (+) sign indicates germination of the spores, while the (–) sign shows failure of germination. Very few (10–20%), moderate (50–60%) and profuse germinations (95% or more) are indicated by a single, double or triple (+) signs respectively.

From Table 13 it is evident that the spores fail to germinate even after 24 hours in media like tap water, and distilled water containing alcohol and lactic acid. Very few spores germinate in pure distilled water after 18 hours, but no further growth of the germ-tubes takes place subsequently. In 1% solutions of glucose and dextrose the spores begin to germinate after 12 hours and the rate of germination is moderate (50–60%). The spores

Table 13. *Rate of spore-germination of Daedalea flavida in different media in hanging-drop cultures and the time required for it*

Media used	Hours							
	3	6	9	12	15	18	21	24
Tapwater	—	—	—	—	—	—	—	—
Distilled water	—	—	—	—	—	—	—	—
Distilled water containing alcohol	—	—	—	—	—	—	—	—
Distilled water containing lactic acid	—	—	—	—	—	—	—	—
1% malt extract soln.	—	+	++	+++	+++	+++	+++	+++
1% sucrose soln.	—	—	—	+	+	++	++	++
1% glucose soln.	—	—	—	+	+	++	++	++
1% dextrose soln.	—	—	—	+	+	++	++	++
2% pure agar	—	—	—	—	—	+	+	+
2% malt-agar	—	+	++	+++	+++	+++	+++	+++
2% dextrose-agar	—	—	—	—	+	+	++	++
Potato-dextrose-agar	—	—	+	+	++	++	+++	+++
Dry spores	+	*	*	*	*	*	*	*

(—) = No germination

(++) = Moderate germination

(+) = Very few germination

(++) = Profuse germination

(*) = Further growth stopped.

germinate readily with speed and vigour, within 6 hours in media like 1% malt extract, and 2% malt-agar. In both the cases germinations after 12 hours are very profuse (more than 95%) giving rise to branched and septate hyphae. Though germination started within 9 hours in *potato-dextrose-agar*, the rate of germination and further growth in this case are slower in comparison with those on 2% malt-agar medium. It is very interesting to note that the spores kept in perfectly dry conditions on slides within Petri dishes, germinate slightly by the formation of short germ-tubes, and this is possibly due to the presence of the 'water-drop' adhering to each spore at its hilum end at the time of spore-discharge. initiating germination of the spore, the 'water-drop' dries up, and as a result no further development of the germ-tube takes place due mainly for want of moisture.

Oxidase tests

Following Bavendamm (1928) oxidase tests were performed, in which the primary and the secondary mycelia of the fungus were allowed to grow separately on 2.5% malt-agar medium containing 0.5% gallic or tannic

acid in Petri dishes. These were then kept in complete darkness at a room temperature of 30–31°C. and a relative humidity of 62%–82%. Dark brown rings appeared around the inocula in all cases, within 24 hours after inoculation showing that *D. flavida* is a 'white-rot' fungus. It was observed that the intensity of reaction was somewhat greater in gallic acid medium than that in the medium containing tannic acid. The results are summarized in Table 14.

Table 14. *Results of oxidase tests with primary and secondary mycelia of Daedalea flavida*

Types of mycelia	Types of reactions in 2.5% malt-agar medium	
	0.5% gallic acid	0.5% tannic acid
Primary mycelium	Very strong (+++); diameter 20 mm.; diffusion zone very intense, dark brown, opaque, forming a wide corona around the inoculum; slight growth above the inoculum (Plate VI, fig. 27).	Strong (+++); diameter 15–18 mm.; other characteristics same as in gallic acid medium (Plate V, fig. 29).
Secondary mycelium	Very strong (+++); diameter 25–28 mm.; diffusion zone very intense, dark brown, opaque, forming a wide corona around the inoculum; no growth of the mat takes place (Plate VI, fig. 28).	Very strong (+++); diameter 18–20 mm.; other characteristics same as those in gallic acid medium (Plate VI, fig. 30).

Cultural characteristics

For studying the cultural characteristics, the isolates from the rotted wood as well as the secondary mycelium were grown on 2.5% malt-agar in Petri dishes. The pH of the medium was adjusted and after sterilization was found to be 6. The cultures in replicates of three for each type of mycelium were incubated in darkness under ordinary conditions of temperature (28–32°C.) and humidity (62%–67%) of the laboratory. Cultural characteristics of both the types of mycelia were found to be identical in every respect.

Growth starts with moderate rapidity, appears somewhat silky over the inocula, and entirely appressed and sodden around them. Within the following 3 days the mycelia become more or less raised and each differentiated into three regions: a thin felty zone around the inoculum, a middle broad sub-felty area, and an appressed and sodden zone of advance. Radial striations over the mats at this stage become evident (PLATE VI, FIG. 25). After about 20 days, a few small, hemispherical or irregularly shaped mycelial lumps develop over the mats. In early stages of development they are white in colour, but with age Warm-Buff and Antimony-yellow colour appear. No further development of these lumps takes place and, in no case, they produce fructifications. The average daily increment in diameter of the mat, in both the cases, has been found to be approximately 11 mm.

An attempt was subsequently made to obtain fruit-bodies in cultures from the isolates and secondary mycelium by growing them on sapwood blocks of *Shorea robusta*. The wood-blocks were prepared and kept in identical conditions, as had been done during decay resistance tests. The fructifications began to appear after 30 days and formed fruitbodies with fertile hymenial surface (PLATE VI, FIG. 26) within 60 days, after inoculation. The details of sporophore production has been communicated in a separate paper.

DISCUSSION

The investigation is an attempt to put on record the part played by *Daedalea flavida* in the annual loss of Sāl wood and some aspects of its life-processes, including the etiological complications which have not so far been well understood.

The decay is entirely restricted to the external sapwood portion of the logs rendering the region somewhat soft and 'brashy'. Decay-resistance tests in the laboratory, however, indicate that the fungus is capable of attacking both sapwood and heartwood under favourable conditions of temperature and humidity. The sapwood is 'perishable' to the attack of the fungus, as it undergoes more than 30% loss in dry weight. The heartwood, on the other hand, suffers more than 10% loss in dry weight, and following Findlay (1938), can be classed as 'non-resistant'. This directly points to the fact that field observations of the gross characters of the rot should not be considered as the sole basis in drawing conclusions, regarding the nature of the rot. The loss in weight of the heartwood blocks in the laboratory test can be safely explained, if one remembers the fact that a moisture-content of the wood above the 'fibre-saturation-point' is necessary for active fungal growth. In the timber-yards generally, this point is not reached, due to huge size of the logs and inadequate water supply, while water has been forced to enter the wood-blocks by exposing them under reduced pressure in the laboratory. It has also been found that the nature and extent of fungal decay depend both on the nature of the wood and the types of mycelia attacking them. The secondary mycelium causes greater amount of decay than the primary mycelium irrespective of the types of wood used. The severity of damage is very acute in sapwood, while the heartwood resists decay within certain limits. Kaufert (1936), working on *Pleurotus corticatus*, obtained similar difference in behaviour of the two types of mycelia as regards their decaying capacity. He was of opinion that this differential behaviour of the two types of mycelia is due to the difference in their rate of growth. As in the present investigation the test period has not been extended beyond four months, no statement can be made at present on the validity of Kaufert's comment on the problem.

The fungus, after infection, ramifies both inter- and intra-cellularly, and easily establishes itself within most of the wood-elements. Enzymic activities of the mycelium undoubtedly render dissolution of some of the components. Eventually, the decay progresses, and cracks appear in the

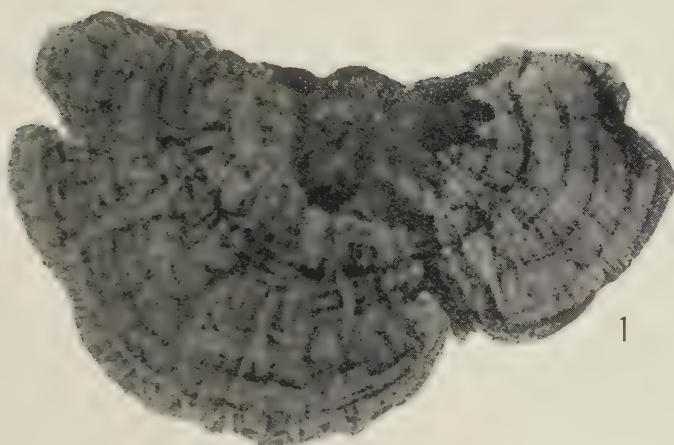
fibrous tissue. Ultimately, the wood loses its toughness, and becomes somewhat soft and compressible. Groups of relatively unaffected fibres break up and render the wood 'brashy'. The enzymic activity of the fungus causes some chemical changes in the wood. Microchemical studies with partially decayed wood show considerable decrease in the amount of lignin. In later stages of decay, however, lignin destruction is still predominant, but the fungus takes up cellulosic materials also. This, perhaps, is due to the fact that, as decay proceeds, more lignin is utilized by the fungus, and as the amount of lignin falls off, the fungus invades cellulosic materials, which are still in abundance, for nourishment. Microchemical studies of the late stages of decay will probably throw some light on this problem, but the thing is beyond the limit, since wood at this stage cannot withstand sectioning and crumbles into 'brashy' needles.

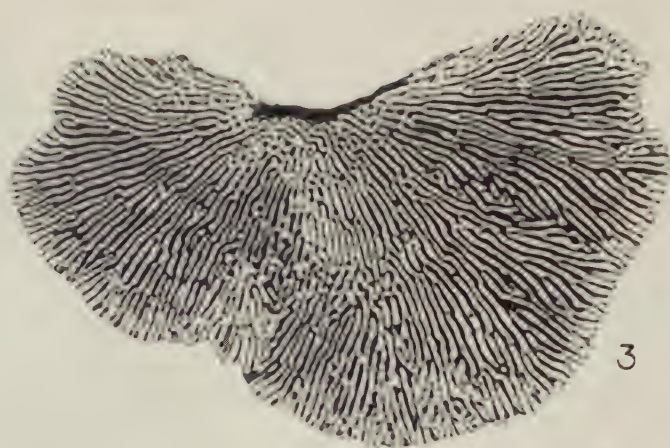
The decomposition of lignin is essentially a process of oxidation, and secretion of oxidases by a 'white rot' fungus is expected. The strong positive reactions of both primary and secondary mycelia with gallic and tannic acid indicate the lignin decomposing nature of the fungus.

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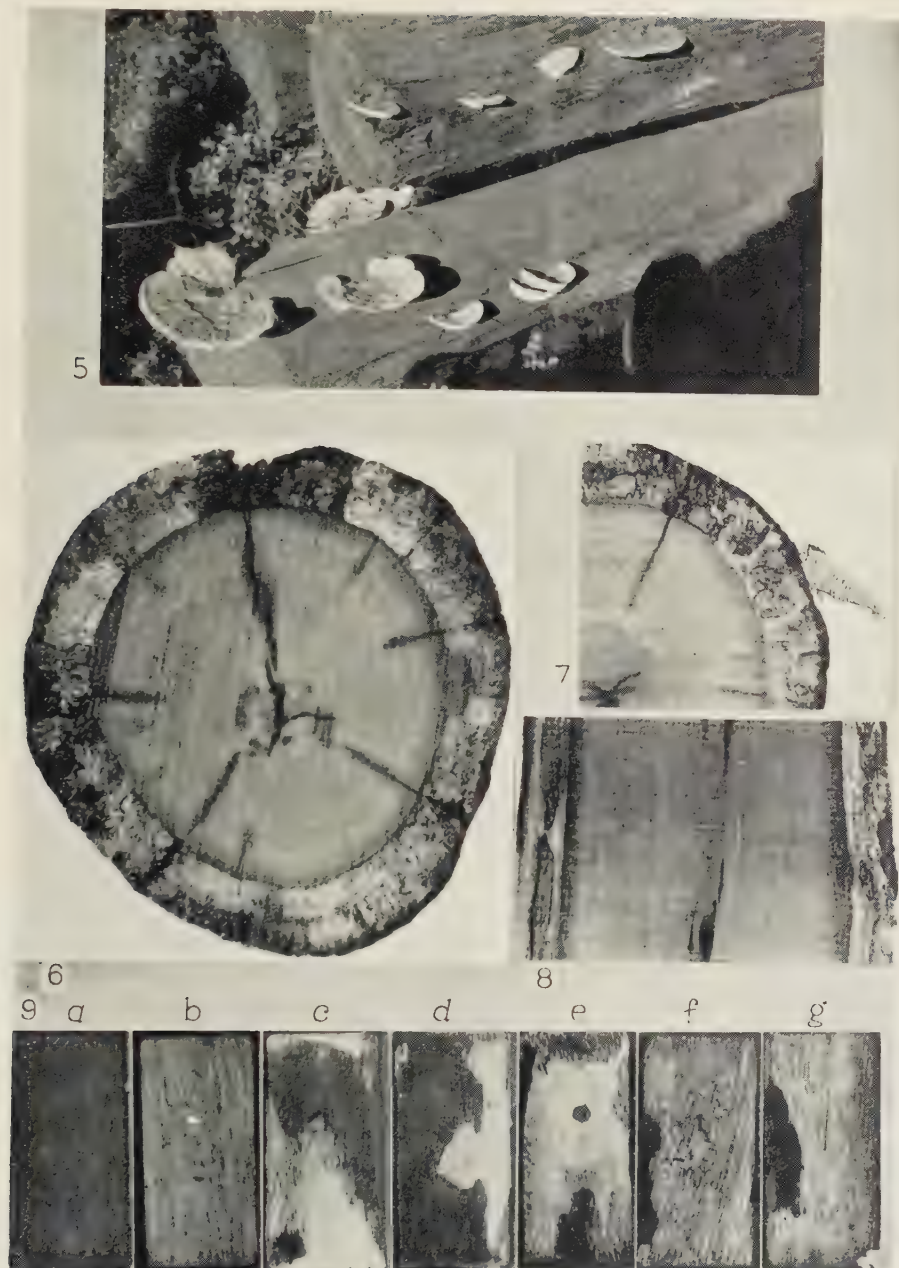
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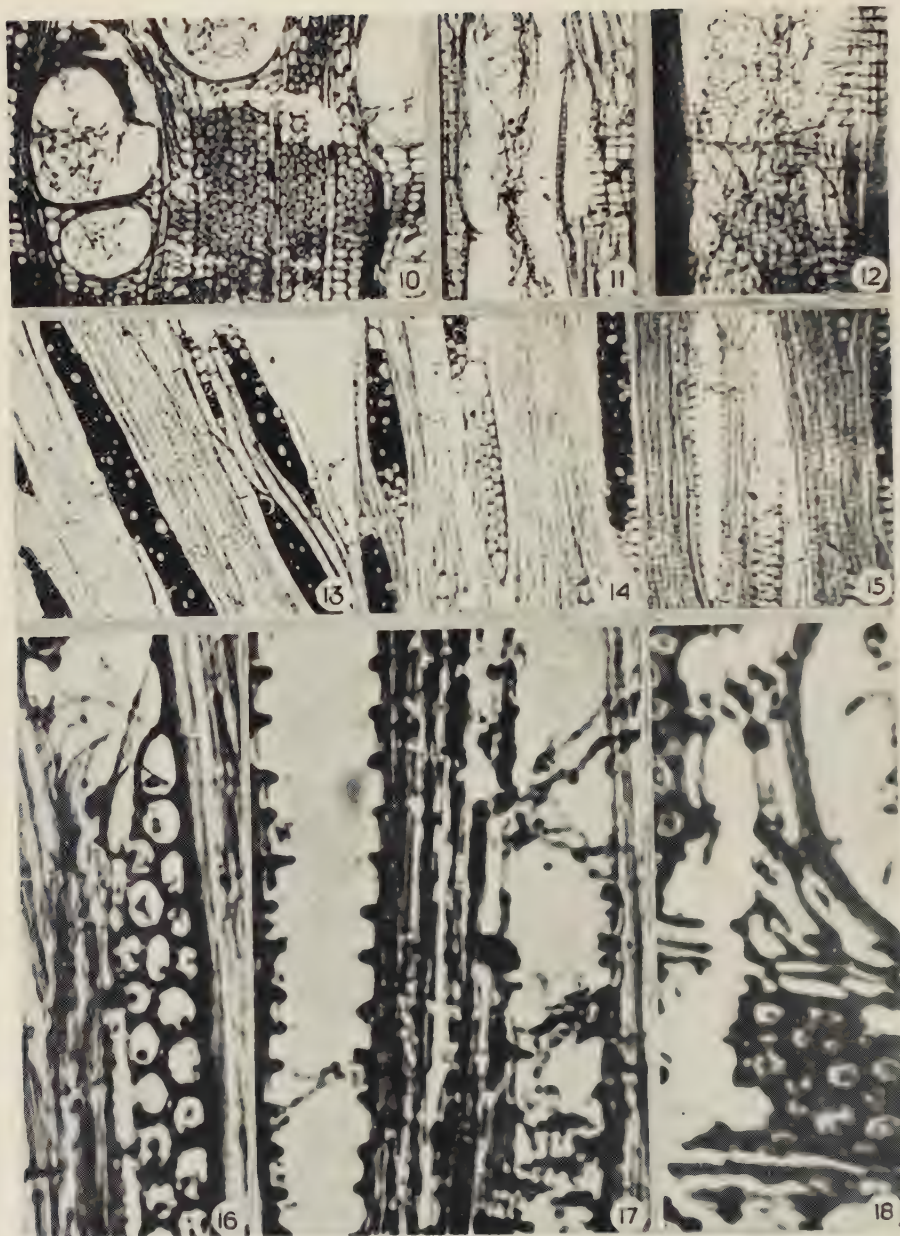
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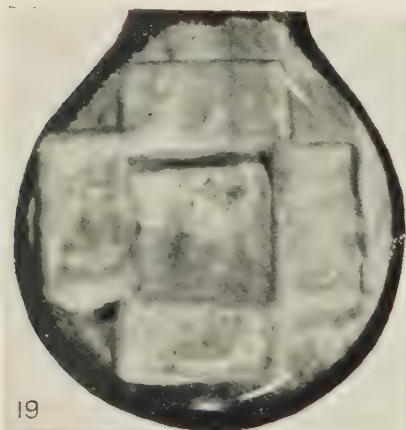
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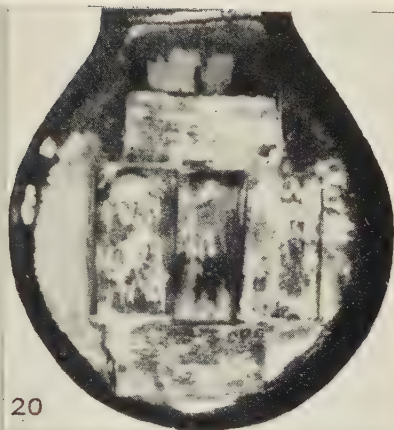
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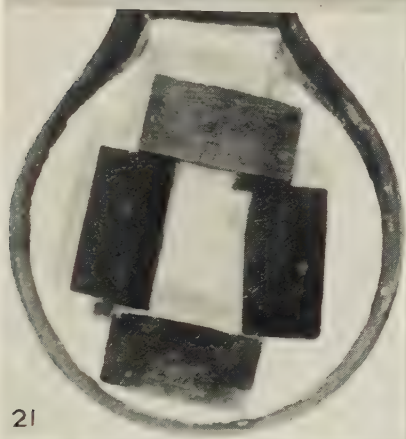




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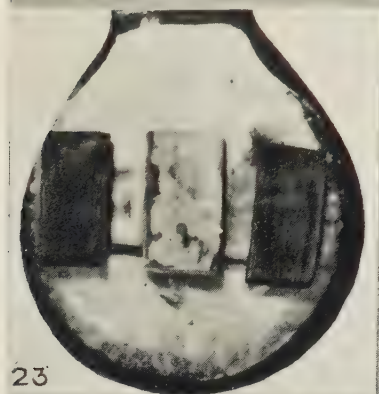
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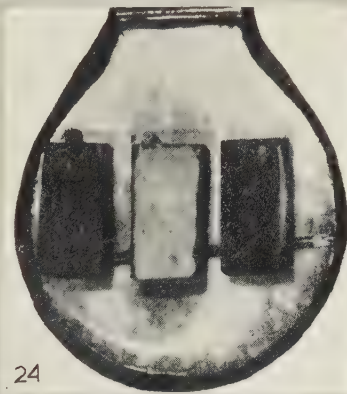
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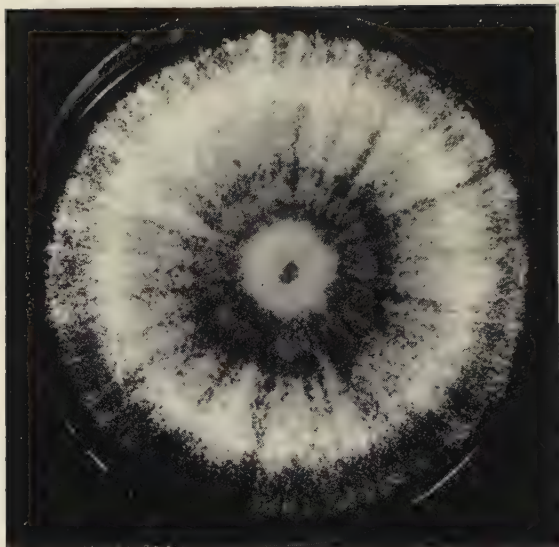
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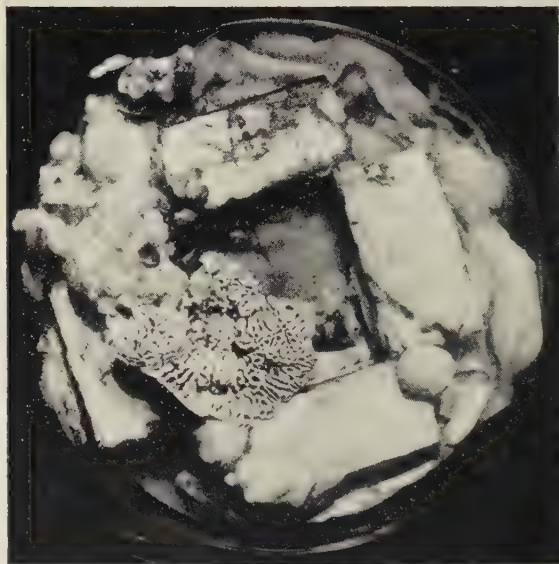
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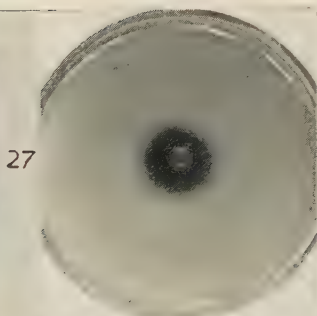
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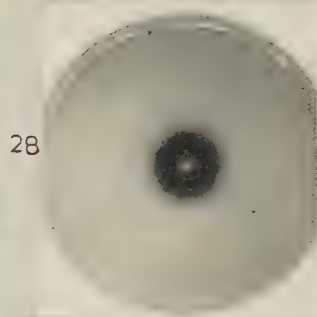
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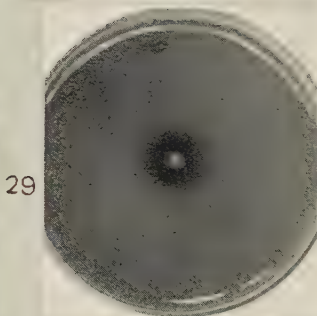
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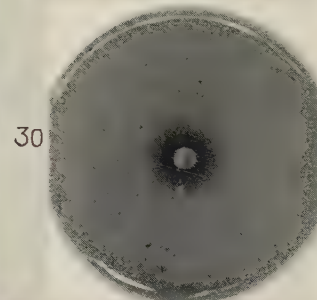
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A Rot of Sal

S. N. Banerjee & K. R. Samadder

EXPLANATION OF PLATES

PLATE I

Figs. 1 & 2. Fructifications of *Daedalea flavida* Lév., showing the upper surface with characteristic zonations.

PLATE II

Figs. 3 & 4. Fructifications of *D. flavida*, showing the hymenial surface with characteristic poroid, labyrinthiform or partly lamellate pore-mouths.

PLATE III

- Fig. 5. Fructifications of *D. flavida* growing both on the cross-section-end and side of Sāl log stacked in a timber-yard.
Fig. 6. Cross section of a log of *Shorea robusta* with sap rot caused by *D. flavida*; the heart-wood portion appears unaffected but with fissures caused due to shrinkage.
Fig. 7. Same type of sap rot with a portion of the basidiocarp of *D. flavida* in contact with the log.
Fig. 8. Part of a longitudinal section of an infected log of *Shorea robusta* showing saprot due to *D. flavida*.
Fig. 9. Test-blocks of sapwood of *Shorea robusta* showing various degrees of bleaching (b-g) due to attack of *D. flavida* after a period of four months' decay-resistance tests; the control test-block (a) does not show such bleaching.

PLATE IV

- Fig. 10. Part of transverse section of sapwood of *Shorea robusta* in an advanced stage of decay with lumina of the vessels filled with the mycelium of *D. flavida*; other elements of the wood are in various stages of disintegration ($\times 80$).
Figs. 11 & 12. Portions of radial-longitudinal sections of sapwood showing distribution of the hyphae within the wood-elements ($\times 80$).
Figs. 13-15. Portions of tangential-longitudinal sections of sapwood showing gradual bleaching and disintegration of the ray cells; hyphal elements can be recognised within the lumina of the ray cells at the centre; dark-coloured gummy materials of the ray cells being gradually used up and finally removed ($\times 80$).
Figs. 16 & 17. Portions of tangential-longitudinal sections of sapwood showing disintegration of the ray cells; hyphal elements are noticeable within lumina of the ray cells, and complete disintegration is evident ($\times 1200$).
Fig. 18. Part of a transverse section of sapwood showing dissolutions of parenchyma cells and eventual crack in the woody tissues ($\times 1200$).

PLATE V

- Figs. 19 & 20. Wood-block cultures of primary (fig. 19) and secondary (fig. 20) mycelia of *D. flavida* growing on sapwood test-pieces; photographed after 30 days.
Figs. 21 & 22. Wood-block cultures of primary (fig. 21) and secondary (fig. 22) mycelia of *D. flavida* growing on heart-wood test-pieces photographed after 30 days.
Figs. 23 & 24. Toxicity test with zinc chloride (fig. 23) and ascu 'A' (fig. 24) respectively in Kolle flasks after a period of four months' test; untreated control test-block in the middle is heavily infected with mycelium, while the treated blocks placed on the two sides of the control remain absolutely free from infection.

PLATE VI

- Fig. 25. 7-days'-old culture of secondary mycelium of *D. flavida* on 2.5% malt-agar.
Fig. 26. Fructification of *D. flavida* formed by an isolate in wood-block culture; the wood-blocks with the fructifications have been carefully taken out from the Kolle flasks and kept on cotton wool in a Petri dish.
Figs. 27 & 28. Oxidase rings around the inocula of primary (fig. 27) and secondary (fig. 28) mycelia of *D. flavida* growing on 2.5% malt-agar containing 0.5% gallic acid.
Figs. 29 & 30. Oxidase rings around the inocula of primary (fig. 29) and secondary (fig. 30) mycelia of *D. flavida* growing on 2.5% malt-agar containing 0.5% tannic acid.

STRAINS OF *COLLETOTRICHUM FALCATUM* WENT IN WEST BENGAL

BY S. B. CHATTOPADHYAY AND ARATI SARKAR

State Agricultural Research Institute, Government of West Bengal

To study the possibility of existence of different strains of *Colletotrichum falcatum* in West Bengal, a large number of isolates were made. They were divided into six distinct morphological groups. Cultural studies on six different media did not show any variation in morphological and growth characters, except that two strains showed slightly lower rate of growth. Pathogenicity studies showed the existence of at least three distinct strains in respect of virulence.

INTRODUCTION

Colletotrichum falcatum Went (Perfect stage, *Glomerella tucumanensis* Arx and Muller), the causal organism of Red-rot disease of sugarcane has been found to be very variable in nature. Existence of strains of different morphological, cultural and pathogenic behaviour has been noted in nature by Abbott (1933) and Chona (1958).

In West Bengal, the Red-rot disease of sugarcane is fairly prevalent, and the disease has been observed to appear with virulence periodically. So, it has been considered necessary to study in detail the existence of strains of *C. falcatum* occurring in West Bengal.

MATERIAL AND METHODS

The fungus was isolated from a large number of infected sugarcane (stems and leaves) collected from time to time from different localities in West Bengal. Small bits of plant tissues taken from affected lesions were dipped in 0.1% Mercuric chloride solution for one minute, washed twice in sterilized distilled water, transferred to *potato-dextrose-agar* slants and incubated at 30°C. The cultures were purified by taking single conidial culture, and the stock cultures were maintained in *potato-dextrose agar* slants at 20°C.

Preliminary cultural study of all the different isolates of *C. falcatum* revealed the presence of six distinct morphological races or strains. These are termed here, for convenience, as A, B, C, D, E and F. Detailed cultural and pathological studies have been undertaken with these strains.

For pathological studies three varieties of sugarcane, viz., Co.313, Co.419 and Co.453 were used. The inoculation experiments were conducted in the laboratory and in the field by adopting "plug" method. The surface of the cane was washed thoroughly with water and then sterilized with rectified spirit. Each cane was then bored in the middle of one internode by inserting a sterilized borer up to the middle and a plug of tissue was taken out. A bit of 10 to 15-days-old culture of *C. falcatum* was then inserted through the bore with a sterile scalpel, and the plug was replaced in position. The place of inoculation was then tied well with moist absorbent cotton. The linear spread of the lesion in each cane was measured by splitting it after 20 days of inoculation.

CULTURAL STUDY

For detailed cultural studies, the strains were grown on six different media, viz., *potato-dextrose-agar*, *potato-sucrose-agar*, *oat-meal-agar*, *cane-leaf-decoction-agar* (Abbott, 1938), *cane-juice-agar* and *Czapek-dox-agar*. For this purpose, Petri dishes (10 cm. diameter), each containing 20 c.cm. of the medium, were inoculated at the centre with mycelial bits of 7-days-old culture from *potato-dextrose-agar* slants. The inoculated Petri dishes were then incubated at 30°–31°C. The diameter of each colony was measured along two directions at right angles to each other. The average growth in diameter was then noted in terms of millimeter. In each case, Petri dishes were used in triplicates and the average reading of the three was taken. Conidial measurements were taken from 15 to 20-days-old cultures. Both length and breadth of the conidia were first measured, but when it appeared that in the breadth there was no appreciable variation, only the length was measured. The average of 100 measurements was recorded in each case.

The distinguishing cultural characters of the *six* strains are given in Table 1.

Table 1. *Cultural characteristics of the strains of C. falcatum at 30°–31°C.*

Strains	Average length of conidia (μ)	Range of conidial length (μ)	Average rate of growth in diameter (cm.)						Cultural characters
			P.D.A.	P.S.A.	O.M.A.	C.L.A.	C.J.A.	C.D.A.	
A	21.8	15.2–26.6	71	74	75	75	78	70	Mat floccose, adpressed at places, ash-grey in colour. Conidial acervuli relatively few.
B	25.8	20.9–32.0	63	63	74	74	63	62	Mat thin and adpressed, light ashy white in colour. Conidia as in A.
C	25.3	20.9–34.2	75	73	75	75	73	73	Mat floccose, cream to ochre coloured. Acervuli with pinkish conidial mass in moderate frequency.
D	27.5	22.8–36.1	67	61	64	63	65	70	Mat floccose, ashy white in colour. Slimy masses of conidia in moderate frequency.
E	22.4	18.0–28.0	74	74	77	78	76	72	Mat dull white, translucent, sparse, cottony. Numerous conidia both in dark grey acervuli and in pinkish masses.
F	23.6	20.0–28.0	70	73	75	77	75	71	Puff-like fluffy mat, dull white in colour with pinkish tinge at places. Conidia mostly in pinkish masses appearing mainly at the centre of the Petri dish.

The strains grew equally well in all the media but variation was noted in the strains B and D, which showed slower rate of growth than the rest. Similar observations of almost equal rate of growth were reported by Abbott (1938), Ramkrishnan (1941), and Chona and Hingorani (1950). The range of spore length lies within the measurements described by Abbott (1938) as 16–48 μ with an average of 25.1 μ .

PATHOGENICITY TESTS

Three varieties of cane, *viz.*, Co.313, Co.419 and Co.453 were selected. Cane cuttings of same size (30" long) and age were taken. They were inoculated in the way described before. For each strain inoculations were performed on 10 cuttings of each variety. Inoculated canes of the same variety were then tied into a bundle and incubated in a moist chamber at 20°C. Separate chambers were used for the bundle of each variety. Cuttings used as control were kept in a separate chamber. They were also treated in the same way except that they were not inoculated with the fungus. After 20 days of incubation the canes were split lengthwise and the lesions were found to spread in all of them but the control remained sound and healthy. Linear spread of the lesions in the affected canes were measured. Re-isolations of all the strains from the respective cane-hosts also confirmed the pathogenicity. The results obtained are given in Table 2.

Table 2. *Data of virulence-tests with cut canes of Co.313, Co.419 and Co.453 varieties inoculated in the laboratory by isolates of six strains of C. falcatum.*

Linear spread of lesions in cm. (average of 10 readings)

Strains	Co.313	Co.419	Co.453
A	13.1	12.0	14.0
B	16.0	13.5	18.4
C	12.2	12.0	11.5
D	14.2	10.5	14.0
E	17.5	16.4	18.5
F	15.0	13.7	18.4

The same experiment was also conducted simultaneously in the field with standing canes of the same three varieties. Plants were inoculated at the age of 6 months. Controls were also maintained properly and 10 replications were set up for each isolate. Two months after inoculation the canes were split lengthwise to expose the lesions due to all the strains. In each case identity of the fungus was confirmed by re-isolation. The controls behaved as in the previous experiment. Linear spread of the lesion measured in terms of centimetre is given in Table 3.

Table 3. *Data of virulence-tests carried on standing canes of Co.313, Co.419 and Co.453 varieties by isolates of six strains of C. falcatum.*

Linear spread of the lesions in cm. (average of 10 readings)

Strains	Co.313	Co.419	Co.453
A	11.7	11.0	11.5
B	14.9	15.0	11.7
C	11.7	12.4	7.0
D	13.9	11.7	13.7
E 	34.7	15.6	33.8
F	18.2	15.4	20.7

Data of these two experiments explain in themselves the pathogenic variation existing among these *six* strains of *C. falcatum*. The strains vary in pathogenicity from a low to a high degree. Taken as a whole, strain A may be regarded as moderately weak, and strain E as the most virulent. Strain F may also be distinguished as less virulent than strain E. The remaining three strains are intermediate in pathogenicity. Variation is also noticed among the different varieties of canes in their susceptibility.

DISCUSSION

All the isolates studied in the present paper belong to the 'light race' of Abbott (1938) who first classified the flora of *C. falcatum* into two morphological groups, *viz.*, 'light race' with profusely sporulating light coloured loose mycelia, and 'dark race' with sparsely sporulating dark compact mycelia. Strains of the light race, in general, were reported to be more virulent than those of the dark race. Chona (1958) also presented similar reports while working with the isolates collected from eastern parts of Uttar Pradesh. No dark race has yet been obtained in the isolates collected in West Bengal, and therefore, comparative studies in regard to virulence of these two morphological groups have not been possible.

It thus appears that altogether *three* virulent strains of *Colletotrichum falcatum*, all belonging to 'light race', are now present in West Bengal.

Further collection of diseased samples and isolation of the Red-rot organism are still in progress, and fresh studies will again be conducted with the new lot of isolates to find out the appearance of any new virulent strain in West Bengal. Moreover, in consideration of the frequent outbreak of the disease in West Bengal, varietal susceptibility tests are also being conducted with a view to finding out resistant varieties of sugarcane suitable for growth in this State.

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CONTRIBUTIONS TO THE CYTOLOGY OF HYMENOMYCETES: IV. CYTOLOGICAL STUDIES IN *LENTINUS SUBNUDUS* BERK. AND *LENTINUS PRAERIGIDUS* BERK.

BY SACHINDRANATH BANERJEE AND SUJATA SANYAL

Department of Botany, University of Calcutta

(with 2 TEXT-FIGURES)

Nuclear phenomena in the life-cycles of *Lentinus subnudus* and *Lentinus praerigidus* have been worked out and found to be in full agreement with their 'heterothallic' and sexually 'bipolar' natures.

Heidenhein's Iron-Alum-Haematoxylin staining yielded satisfactory preparations when used after fixation with standard killing and fixing fluids.

In both the species each basidiospore, when first formed, receives only one nucleus which prior to germination may or may not divide again. It germinates to produce a septate primary mycelium of uninucleate cells and without clamp-connexions.

Secondary mycelia, of both binucleate (conjugate pair) and multinucleate hyphal cells, in both the species are obtained by pairing, compatible primary mycelia. The clamp-connexions, however, occur much infrequently.

In both the species, the young basidium is always binucleate and the nuclei soon fuse to form a large fusion-nucleus. This fusion-nucleus divides reductionally to produce four haploid nuclei. The orientation of the first-division-spindle is transverse in *L. subnudus* and longitudinal in *L. praerigidus* while that of the spindles during second-division is extremely variable. Each of the tetracyte nuclei finally enters a spore. The basidium, after spore-discharge, becomes empty and gradually collapses.

The haploid and diploid chromosome numbers in both the species have been found to be 3 and 6 respectively.

INTRODUCTION

In the past quite a large number of Agaricaceae have been cytologically investigated by Sass (1929), Wakayama (1930) and others, and Olive (1953) has given an excellent review on the subject. From the available records it is evident that, so far as the genus *Lentinus* is concerned, very little cytological details are known, *Lentinus variabilis* being the only species which has been investigated by Kühner (1928). The present communication reports on the karyological phenomena in the life-cycles of *Lentinus subnudus* Berk. and *Lentinus praerigidus* Berk., the two noteworthy species of Agaricaceae of subtropical India, and forms a part of the series of investigations taken up by Banerjee and his co-workers (1955, 1956).

In conformity with the modern tendency of seeking a cytological basis of the sexual behaviour of Hymenomycetes, the object of this investigation, apart from the karyological study of the life-cycles in the main, is to look for a correlation of the data obtained by the writers (1956) during their studies on heterothallism.

MATERIAL AND METHODS

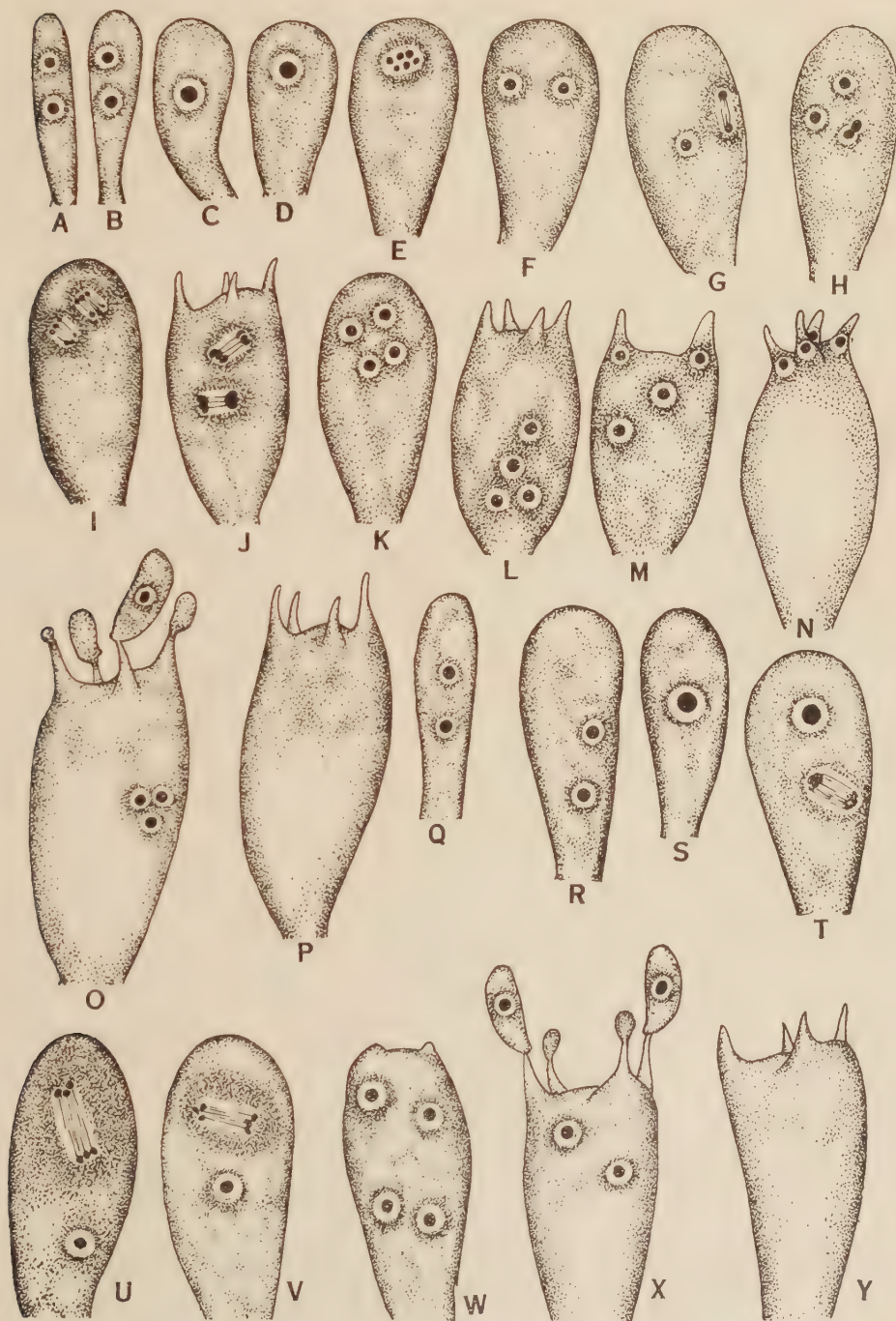
For studying the nuclear conditions in basidia, fresh fruit-bodies of both the fungi were fixed in several fixing fluids at different intervals of time between 1 to 3 a.m. The fixing fluids used were 'Flemming' (weak and strong), 'La Cour (2BD)', 'Bouin-Allen', 'Sass', 'Chromo-Acetic acid', 'Formal-Acetic-Alcohol (weak and strong), and 'Navaschin (A and B)'. Initial fixation was done under reduced atmospheric pressure to facilitate rapid penetration of the fixing fluids. After fixation washing, dehydration and embedding were done following the standard alcohol-chloroform schedule. Microtome sections, 8μ in thickness, were found to be suitable for observations. Several stains, such as Heidenheim's Iron-Alum-Haematoxylin, Crystal Violet (1% aq. soln.) and aqueous Basic Fuchsin, were tried. The first one yielded very satisfactory result when used after fixation with 'Flemming (Strong)' or 'La Cour (2BD)'.

In order to study the karyological conditions in the spores, germinating spores and primary and secondary mycelia, Kniep's (1913) agar-film technique and that modified by Banerjee (1958) were employed. Spore-deposits were directly taken on thin films of cleared 2% *malt-agar* on sterile slides. Preparations for spore-cytology were immediately fixed in all the fixatives mentioned above, while those for germinating stages were incubated at room temperature (28–30°C.) and fixed when the desired stages of germination were obtained. Total preparations of primary and secondary mycelia were also prepared. The nuclei of the spores and germinating spores were most satisfactorily fixed with 'Chromo-Acetic acid' and 'Sass'. 'Flemming (weak)' and 'Chromo-Acetic acid', on the other hand, gave best results for primary and secondary mycelia. In all these preparations Iron-Alum-Haematoxylin proved to be the most satisfactory one.

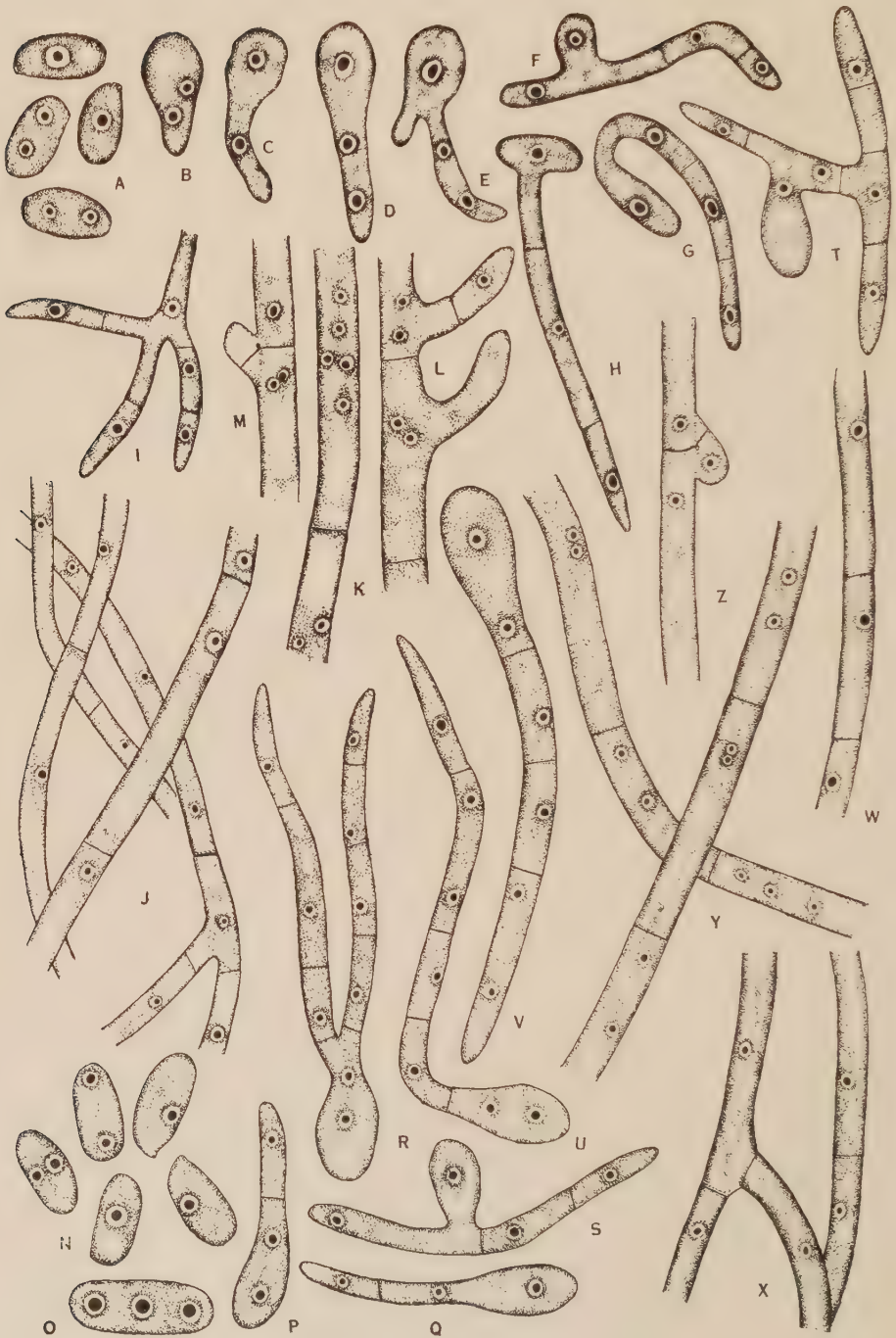
OBSERVATIONS

Lentinus subnudus Berk.

At maturity, each basidium is broadly clavate, slightly wider than the terminal hyphal cells, contains two nuclei about half-way up the basidium and lying one above the other in the general mass of cytoplasm (TEXT-FIG. 1, A & B). This conjugate condition soon comes to an end and karyogamy occurs to produce a synkaryon which gradually moves towards the upper part of the basidium (TEXT-FIG. 1, C & D). The synkaryon at this stage is conspicuously large, compact, homogenous and well-defined. Soon meiosis occurs but the details of early prophase have not been clearly observed in the preparations. The chromosomes, however, have been clearly demonstrated in first division metaphase and can be counted with sufficient accuracy ($2n = 6$: TEXT-FIG. 1, E). The first division spindles are intranuclear in origin and of chiasmatobasidial type in orientation. After the first division the two nuclei (TEXT-FIG. 1, F) divide either simultaneously (TEXT-FIG. 1, I & J) or in succession (TEXT-FIG. 1, G & H). The spindles are usually obliquely placed and are often parallel to each other (TEXT-FIG. 1, I & J). In some cases they are longitudinal (TEXT-FIG. 1, G) or even transverse (TEXT-FIG. 1, J). No centrosomes



TEXT-FIG. 1. Nuclear phenomena in the basidia of *L. subnudus* (A—P) and *L. praerigidus* (Q—Y). $\times 3000$. (For explanation vide text).



TEXT-FIG. 2. Nuclear phenomena in the basidiospores, germinating basidiospores and mycelia (primary and secondary) of *L. subnudus* (A—M) and *L. praerigidus* (N—Z). $\times 1500$. (For explanations vide text).

have been observed in any stage of meiosis. After the completion of meiosis four nuclei are formed, which are all alike in size and staining properties. Their orientation within the basidium is extremely variable (TEXT-FIG. 1, K-M) and this is a striking feature of the basidial nuclei in this species. Before or after the completion of meiosis four conical sterigmata begin to develop on each basidium and a spore is eventually formed at the apex of each sterigma. As the sterigmata and the spores develop, the nuclei move upwards (TEXT-FIG. 1, M & N) within the basidium and finally migrate through the sterigmata into the spores (TEXT-FIG. 1, N). Each spore thus receives a single nucleus. The spores are formed in succession (TEXT-FIG. 1, O) and the migration of the nuclei towards the top of the basidium is either simultaneous (TEXT-FIG. 1, N) or in succession (TEXT-FIG. 1, O). After the formation and discharge of the four spores the basidium becomes empty (TEXT-FIG. 1, P).

Mature spores, although in great majority of cases uninucleate, occasionally contain two nuclei (TEXT-FIG. 2, A). This is probably due to division of the pre-existing nucleus. Germination of the spores takes place at this binucleate stage by the formation of a germ-tube from any point of the spore-wall into which one of the two nuclei migrates (TEXT-FIG. 2, B & C). Frequently two germ-tubes may develop from the same place on the spore-wall but one of them soon takes the lead (TEXT-FIG. 2, E & F). The first migrated nucleus divides again to produce two nuclei (TEXT-FIG. 2, D & E) and these are eventually separated from the spore by the formation of a transverse septum (TEXT-FIG. 2, F-H) or the septum formation may be delayed (TEXT-FIG. 2, D & E, I). Further elongation of the terminal cell of the germ-tube is followed by nuclear division and wall-formation in succession, so that, after branching a mycelium of uninucleate cells is eventually formed (TEXT-FIG. 2, G-J). This is the condition of the primary mycelium in the advanced stage and the clamp-connexions are entirely absent (TEXT-FIG. 2, J). Cells of the secondary mycelium, on the other hand, are typically binucleate although three to five nuclei with plane septa are not uncommon (TEXT-FIG. 2, L & K). The occurrence of clamp connexions in the secondary mycelium is not regular. The same hypha may bear plane septa as well as clamp-connexions (TEXT-FIG. 2, M).

Lentinus praerigidus Berk.

Young basidia are distinctly binucleate. The two nuclei lie half-way up the basidium and a little distance apart from each other (TEXT-FIG. 1, Q & R). Karyogamy follows and a Synkaryon is formed. The Synkaryon is conspicuously large and occupies the middle position of the basidium (TEXT-FIG. 1, S). First division is stigobasidial as can be assumed from the position of the two resulting daughter nuclei (TEXT-FIG. 1, T). Second division of meiosis takes place in succession and the spindles appear to be somewhat obliquely placed (TEXT-FIG. 1, T & U). The number of chromosomes at each pole of the spindle of the anaphase during second division has been found to be $n = 3$ (TEXT-FIG. 1, U & V). Spindles are intranuclear in origin and no centrosomes have been found in any of the preparations. After the formation of 4 daughter nuclei (TEXT-FIG. 1, W), four sterigmata begin to develop at the apex of each basidium and four

spores are ultimately formed at the apices of the sterigmata. The development of the spores is not simultaneous but they are produced in succession (TEXT-FIG. 1, X). Each spore receives a single nucleus from the basidium and after the discharge of the spores the basidium becomes empty (TEXT-FIG. 1, Y).

Mature spores are mostly uninucleate although occasionally 2 to 3 nuclei in each spore have been observed (TEXT-FIG. 2, N & O). Trinucleate condition is rare, and when it appears the spore becomes somewhat enlarged. When the germination starts the spore-nucleus divides into two daughter nuclei. A germ-tube is formed at either end of the spore into which one of the daughter nuclei migrates, completes another mitosis and a transverse septum is formed in between the two in such a way that a terminal uninucleate cell is formed in the germ-tube while the other two nuclei remain within original spore-case or one towards the base of the germ-tube and the other within the spore-case (TEXT-FIG. 2, P & Q). Sometimes two germ-tubes develop from the same point of origin on the spore-wall and as they develop they grow side by side more or less parallel to each other (TEXT-FIG. 2, R) or in opposite directions (TEXT-FIG. 2, S & T). The terminal cell of the germ-tube then elongates, its nucleus divides, septum-formation follows and this process is repeated so that eventually a mycelium consisting of uninucleate cells is formed (TEXT-FIG. 2, T-X). The cells of the primary mycelium is decidedly uninucleate and lack clamp-connexions (TEXT-FIG. 2, W & X) while the cells of the secondary mycelium is typically binucleate although three to four nuclei with plane septa are not uncommon (TEXT-FIG. 2, Y). The occurrence of clamp-connexions in the secondary mycelium is, as in the case of *Lentinus subnudus*, not regular and are somewhat infrequent (TEXT-FIG. 2, Z).

DISCUSSION

Nuclear phenomenon in the life-cycle of the two fungi can be considered as corroborative evidences to their heterothallic nature. On the basis of karyological conditions, the distribution of sex-factors and the consequent sex-reactions in these two fungi can be explained. In both the fungi the basidiospores are at first uninucleate but the nucleus of the spore always divides into 2 or 3 nuclei at the time of germination and gives rise to a primary haploid mycelium of uninucleate cells. When primary mycelia of opposite sexes meet they form secondary mycelium with clamp-connexions. The number of dicaryons in each hyphal cell is usually one but three or more nuclei may be present in each cell. The occurrence of clamp-connexions is not regular and the same mycelium may have both clamp-connexions and plane septa. This shows that the conjugate pair can divide independently. This binucleate condition comes to an end with the formation of basidia in each of which the two nuclei fuse to form a synkaryon. The synkaryon divides reductionally to form four haploid nuclei. Since both the species are 'bipolar' segregation takes place during the first division of the synkaryon. The cytological data again suggest the way in which the tetracyte nuclei of the basidium are distributed to form four haploid basidiospores. The nuclei are all of 'homogenous' type

of Pinto-Lopes (1949) in which the chromatin and nucleolus being contracted to an intensely stainable body and the major part of nucleoplasm is free from chromatin. Since the chromosomes are too small, the details of their karyotype is out of the scope. Chromosome number, however, have been demonstrated with sufficient clearness and has been found to be $2n = 6$ in both the cases. Maire (1902), Levine (1913) and others have shown centrosomes and astral rays in meiosis but no such centrosomes or astral rays are visible in any of the preparations of the writers. The variable distribution of the tetracyte nuclei of the basidium is an interesting phenomenon. Further, the orientation of the spindle in the first division of the fusion nucleus is transverse in *L. subnudus* and probably longitudinal in *L. praerigidus* while that of the spindles during second division is extremely variable. In any case the spindles are intranuclear in origin.

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SEXUALITY OF *TRAMETES CINGULATA* BERK.

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MATING experiments with fifteen primary mycelia of monosporous origin have revealed that *Trametes cingulata* Berk. is 'heterothallic' and sexually 'tetraplar'. Four principal types of reactions, namely, 'compatible', 'neutral', 'antagonistic' and 'inhibitory' have been noticed during pairings of primary mycelia and these have been described in detail. A compatible pair always produces a secondary mycelium showing clamp-connexions.

INTRODUCTION

Investigations on the phenomenon of heterothallism in Basidiomycetes are steadily increasing in India, and in this laboratory have been carried out on a number of species by Banerjee and his co-workers (1954, 1955, 1956a, 1956b, 1957, 1958). *Trametes cingulata* Berk. is another fungus of the series under study. *Trametes cingulata* is a common wood-destroying Polyporaceae of tropical and subtropical India. It is evident from the available literature (Whitehouse, 1949; Quintanilha and Pinto-Lopes, 1950; Raper, 1953) that no work has so far been done on the sexual phenomenon of this fungus. An attempt has, therefore, been made to throw some light on the nature of heterothallism existing in *Trametes cingulata*.

MATERIAL AND METHODS

With a view to determine the sexual behaviour of *Trametes cingulata* fifteen monosporous mycelia were derived from a single sporophore. The surface of a cleared, filtered and sterile agar in a Petri dish was flooded with a dilute spore-suspension in sterile distilled water and allowed to stand for a few minutes. The water was then poured off. In this way, several other plates were also prepared. These were then incubated at room temperature (30°C.) for 10 to 12 hours and finally examined. The lid of the Petri dish was removed and well-germinated and isolated spores were then punched out as small agar discs, each containing a single germinating spore, by means of a sterile cutter and transferred to 2.5% malt-agar slants. In all cases, mycelia developed within the tubes and after careful examination they were all found to be in the primary condition and without clamp-connexions.

Fifteen monosporous cultures were then paired in all possible ways on 2.5% malt-agar slants (pH 6) in culture tubes and were allowed to grow for a month. At the end of the period, the line of contact between the paired mycelia as well as the unpaired ones was carefully examined for the presence of clamp-connexions. The results of these examinations are recorded in the Tables 1 and 2.

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Table 1. Pairings of 15 monosporous mycelia derived a single sporophore of *Trametes cingulata*

	AB				ab				Ab				aB			
	1	4	10	15	3	7	11	12	2	6	9	13	14	5	8	
AB	1	-	-	-	+	+	+	+	-	-	-	-	-	-	-	
	4	-	-	-	+	+	+	+	-	-	-	-	-	-	-	
	10	-	-	-	+	+	+	+	-	-	-	-	-	-	-	
	15	-	-	-	+	+	+	+	-	-	-	-	-	-	-	
ab	3	+	+	+	+	-	-	-	-	-	-	-	-	-	-	
	7	+	+	+	+	-	-	-	-	-	-	-	-	-	-	
	11	+	+	+	+	-	-	-	-	-	-	-	-	-	-	
	12	+	+	+	+	-	-	-	-	-	-	-	-	-	-	
Ab	2	-	-	-	-	-	-	-	-	-	-	-	-	+	+	
	6	-	-	-	-	-	-	-	-	-	-	-	-	+	+	
	9	-	-	-	-	-	-	-	-	-	-	-	-	+	+	
	13	-	-	-	-	-	-	-	-	-	-	-	-	+	+	
aB	14	-	-	-	-	-	-	-	-	-	-	-	-	+	+	
	5	-	-	-	-	-	-	-	+	+	+	+	+	-	-	
	8	-	-	-	-	-	-	-	+	+	+	+	+	-	-	

(+) — Clamp-connexion
(-) — No clamp-connexion

Table 2. Pairings of 15 monosporous mycelia derived from a single sporophore of *Trametes cingulata* showing different types of reactions.

	AB				ab				Ab				aB			
	1	4	10	15	3	7	11	12	2	6	9	13	14	5	8	
AB	1	N	N	N	N	C	C	C	C	I	I	I	I	A	A	
	4	N	N	N	N	C	C	C	C	I	I	I	I	A	A	
	10	N	N	N	N	C	C	C	C	I	I	I	I	A	A	
	15	N	N	N	N	C	C	C	C	I	I	I	I	A	A	
ab	3	C	C	C	C	N	N	N	N	A	A	A	A	I	I	
	7	C	C	C	C	N	N	N	N	A	A	A	A	I	I	
	11	C	C	C	C	N	N	N	N	A	A	A	A	I	I	
	12	C	C	C	C	N	N	N	N	A	A	A	A	I	I	
Ab	2	I	I	I	I	A	A	A	A	N	N	N	N	C	C	
	6	I	I	I	I	A	A	A	A	N	N	N	N	C	C	
	9	I	I	I	I	A	A	A	A	N	N	N	N	C	C	
	13	I	I	I	I	A	A	A	A	N	N	N	N	C	C	
aB	14	I	I	I	I	A	A	A	A	N	N	N	N	C	C	
	5	A	A	A	A	I	I	I	I	C	C	C	C	N	N	
	8	A	A	A	A	I	I	I	I	C	C	C	C	N	N	

N — Neutral
C — Compatible
A — Antagonistic
I — Inhibitory

RESULTS

From the Tables it will be observed that mycelia 1, 4, 10 and 15 paired with mycelia 3, 7, 11 and 12 and mycelia 2, 6, 9, 13 and 14 paired with mycelia 5 and 8 in such a way that they formed clamp-connexions; but other combinations exhibited 'neutral', 'inhibitory' and 'antagonistic' behaviours. This indicates that *Trametes cingulata* is 'heterothallic' and exhibits 'tetrapolar' type of sexuality. The four groups can be designated as AB, ab, Ab and aB. The mycelia 1, 4, 10 and 15 (AB) are complementary and compatible with mycelia 3, 7, 11 and 12 (ab). Again, the mycelia 2, 6, 9, 13 and 14 (Ab) are compatible and complementary with mycelia 5 and 8 (aB).

In all, four principal types of reactions are noticed from the interactions of primary mycelia of *Trametes cingulata* as stated below :

Compatible—Two primary mycelia intermingle freely, eventually lose their identity, and secondary mycelium with clamp-connexions is formed at the line of contact which tends to grow upwards forming a raised mat. There is no line of aversion and the agar medium is not discoloured below their place of contact.

Neutral—Two primary mycelia merge into one another forming a homogeneous mat. The secondary mycelium with clamp-connexions is not formed. The medium below the mat is also not discoloured.

Antagonistic—Two primary mycelia meet to form a distinct line of aversion separating the two interacting colonies. In some cases, however, a narrow space of aversion is formed and this space is filled with their intermingling mycelia. Neither secondary mycelia nor clamp-connexions are present. A dark zone in the agar medium is formed just below the line of separation.

Inhibitory—Two primary mycelia remain separated from each other and appear to be antagonistic in nature, bearing a clear wide space between them. As there is no intermingling between the interacting hyphae, secondary mycelium with clamp-connexions cannot be formed. There is also discolouration of the medium just below the separation zone.

DISCUSSION

From the behaviour of the interacting primary mycelia it is clear that *Trametes cingulata* shows tetrapolar type of sexuality. In a tetrapolar species the mycelia derived from the four spores of a basidium belong to four different sex groups. It has been assumed that in this case there are two allelomorphic pairs of sex factors in the fusion nucleus of a basidium and genetically four different types of nuclei are derived due to reduction division of the synkaryon in the basidium, that is, the tetrapolar species are able to pair in such a way as to form dykaryotic mycelia due to the "occurrence and independent assortment of allelomorphic determining factors at the two genetic loci". These factors are designated as *A*, *a* and *B*, *b*. As a result of reduction division of the synkaryon, four daughter nuclei are derived which may have the genetic constitution as *AB*, *Ab*, *aB* and *ab*. Matings between mycelia forming dikaryotic stage will only take place between complements so as to restore a double heterozygote, namely, $AB \times ab$ and $Ab \times aB$.

The behaviour of such heterothallic forms is unique in the sense that the combinations of strains, such as, $AB \times Ab$, $aB \times ab$, $AB \times aB$ or $Ab \times ab$, fail to produce the typical heterozygote for the initiation of dikaryon. Here are observed two distinct kinds of reactions which have been described as 'antagonism' and 'inhibition'. Inhibition may be defined as an extreme case of antagonism leaving a wide space of aversion between them. In a typical example of antagonism where similar incompatibility between interacting mycelia is observed, the space of aversion is absent or greatly reduced. This difference in the behaviour of the incompatible mycelia may have the following cytological explanation. The combination of strains where heterozygosity (*ABab*) is disturbed due to the presence of the same factor in allelomorphs (*A* or *a*) cause inhibition as in $AB \times Ab$ or $aB \times ab$. On the other hand, the combinations where heterozygosity is disturbed due to the presence of the factor *B* or *b*, the result is antagonism as in $AB \times aB$ or $Ab \times ab$.

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STUDIES ON *IRPEX FLAVUS* KL. —I
ANTAGONISM BETWEEN DIFFERENT STRAINS
AND THEIR HOST-RELATIONS

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(with PLATES I-II and 2 TEXT-FIGURES)

The strains of *Irpex flavus* obtained from *Shorea robusta* Gaertn.f., *Swietenia mahagoni* Linn. and *Albizzia lebbek* Benth., have been paired in all possible combinations with a view to find out the types of reaction existing between the interacting mycelia. Incidentally, the host-relations of the strains have also been studied on the basis of their relative variation in decay.

The fruit-bodies collected from the aforesaid hosts are morphologically more or less similar. The antagonistic effect between the interacting strains, irrespective of the types of mycelia (monosporous and polysporous mycelia or the isolates), has been noticed in all the pairings excepting between the subcultures of the same mycelium.

It appears from the cross-inoculation experiments that the variation in the average percentage of loss in dry weights of each host-species is dependent on the types of mycelia and also on the different strains of the same type of mycelium. But the strains under consideration show their maximum ability to decay in case of *Albizzia*, minimum in *Shorea* and moderate in *Swietenia* and hence *Albizzia* may be considered as 'least resistant' among the three hosts while *Shorea* is 'most resistant' and the remaining one may be placed in the intermediate category.

INTRODUCTION

Irpex flavus Kl., a common wood-rotting fungus in India, attacks our structural timbers, such as *Shorea robusta* Gaertn.f., *Swietenia mahagoni* Linn., *Albizzia lebbek* Benth. and several other hard-wood species and monocotyledons causing decay of large quantities of wood annually. Besides India, it also occurs in the warmer countries of the world. Its effuso-reflexed, coriaceous, yellowish fruit-bodies with dentate hymenial surface, so common on logs, stumps and dead trunks of trees, provide characteristic features by which it can be readily recognized in the field. Owing to its frequency of occurrence, many citations are made to this fungus, but strangely enough no intensive studies have so far been undertaken on this species. Since the host-species of *Irpex flavus* are of great economic importance, it is felt that any information concerning the fungus is desirable. With this end in view, investigations have been undertaken and the present paper forms the first contribution of the series.

This investigation comprises two main lines of inquiry. Firstly, pairing experiments have been carried out in order to study the interactions between the strains obtained from *S. robusta*, *S. mahagoni* and *A. lebbek*. Secondly, cross-inoculation-tests of the sapwood of the aforesaid hosts with the different isolates and monosporous mycelia have been performed under a set of controlled conditions in order to establish host-relations of the available strains under consideration.

MATERIAL AND METHODS

For this investigation three economic timbers, viz., *Shorea robusta*, *Swietenia mahagoni* and *Albizzia lebbek* were selected and obtained from different timber-yards of Calcutta in the months of August to November, 1957. Short cylindrical cross-sections of the poles of the three types of infected logs were brought to the laboratory, examined and studied in a fresh condition. Plenty of fructifications of *Irpex flavus* were also collected separately from the different hosts. From each type of fruit-body copious spore-deposits were obtained on 2 per cent sterile agar plates within few hours after collections and from these spores polysporous cultures were made on 2.5 per cent *malt-agar* slants. Under ordinary conditions of temperature (30°-32°C.) and diffused light of the laboratory, the spores germinated and the resulting mycelia covered the surface of the slants within a week. In this way polysporous cultures of different strains were obtained. Similarly, monosporous cultures from the spores obtained from the different fruit-bodies were prepared by the usual dilution method (Smith, 1954). The mycelia from three different hosts were also isolated on 2.5 per cent *malt-agar* following the method adopted by Banerjee (1955). Out of thirty-six isolations from the different regions of sapwood and heartwood blocks of the three hosts, pure cultures were obtained in twenty-four cases. In some cultures fructifications began to develop within a fortnight. These cultures were then compared with monosporous and polysporous cultures of the respective strains and found to be identical as regards their macroscopic appearance and microscopic details. It may be worthwhile to note that clamp-connexions were absent in all cases.

Antagonism

With a view to determine the types of reaction between the strains of *Irpex flavus* obtained from different hosts, pairing experiments have been undertaken. Several workers like Schmitz (1925), Verrall (1934) and others have made notable contributions on other fungi in this line. It has been found that antagonism exists between the strains obtained from different host-species. Though similar antagonistic behaviour has been noticed in certain cases of heterothallic species yet it is not uncommon in other cases also. For the present investigation both monosporous and polysporous mycelia have been obtained from fruit-bodies on *Shorea robusta*, *Swietenia mahagoni* and *Albizzia lebbek* and different isolates have also been taken from the afore-said hosts. These have then been paired with the presumption, that if there be any variation between the strains, there is every possibility of the existence of antagonistic reaction among themselves irrespective of the types of mycelia and the isolates.

Schmitz (1925) while working with *Fomes pinicola* paired the cultures from four different hosts, namely, Douglas fir, white fir, western hemlock and western white pine and found no intermingling among themselves. However, according to him the line of demarcation found between two monosporous and polysporous mycelia of *Fomes pinicola* was probably due to physiological variation existing in the mycelia and that caused an upset in the metabolism when the two cultures met. The maximum metabolic change was indicated by the presence of a dense line of demarcation between them.

Similarly, Verrall (1934) worked with *Fomes igniarius*. He also paired the cultures from different hosts and came to the conclusion that when the isolates from the different hosts were paired, irrespective of their similarity or difference, there would be antagonism. In case of haploid cultures, he showed two types of interactions, one with 'no sexual fusion' and other with 'sexual fusion'. Under the type 'no sexual fusion' he pointed out that all haploid lines from different hosts and all haploid lines of the same sex would show this type of reaction. In such cases demarcation line would be formed between the paired cultures and the subculture from the line of demarcation might represent the original cultures by separation.

In case of *Irpex flavus*, aforesaid types of mycelia were paired in possible combination in Petri dishes (100 mm.) containing 2% cleared malt-agar and kept at ordinary temperature (27°-32°C.), humidity (66%-96%) and diffused light of the laboratory. After four days of inoculation the line of contact of the pairings of different monosporous and polysporous mycelia and the isolates were examined directly under the microscope. The hyphae of the advancing mycelia were found to overlap each other but so far as the observations go, no hyphal fusion was found in any case (PL. I., FIGS. 1-3). Gradually, a dense white demarcation line appeared in all cases between the interacting mats within a week (PL. II., FIGS. 5-7). These white lines ultimately became dark coloured in the majority of the cases (PL. II., FIGS. 8-10). Furthermore, subcultures from the line of demarcation also produced two separate mats within a fortnight (PL. I., FIG. 4.)

The results of pairing experiments are given in Tables 1-3.

Table 1. *Pairings of three different monosporous mycelia of Irpex flavus obtained from the fruit-bodies from three different hosts.*

	Sm	Mm	Am
Sm	C	A	A
Mm	A	C	A
Am	A	A	C

Sm = Monosporous mycelium from *Shorea robusta*

Mm = Monosporous mycelium from *Swietenia mahagoni*

Am = Monosporous mycelium from *Albizia lebbek*

A = Antagonistic

C = Compatible

Table 2. Pairings of three different polysporous mycelia of *Irpex flavus* from three different hosts.

	Sp	Mp	Ap
Sp	C	A	A
Mp	A	C	A
Ap	A	A	C

Sp = Polysporous mycelia from *Shorea robusta*Mp = Polysporous mycelia from *Swietenia mahagoni*Ap = Polysporous mycelia from *Albizzia lebbek*

A = Antagonistic

C = Compatible

Table 3. Pairings of three different isolates of *Irpex flavus* from three different hosts.

	Si	Mi	Ai
Si	C	A	A
Mi	A	C	A
Ai	A	A	C

Si = Isolate from *Shorea robusta*Mi = Isolate from *Swietenia mahagoni*Ai = Isolate from *Albizzia lebbek*

A = Antagonistic

C = Compatible

From the foregoing tables, it will be evident that antagonism occurs in every case except when a mycelium is paired with its own subculture. In the latter case, the interacting mycelia intermingle freely showing clear hyphal fusions between them and there is no line of aversion. These interacting subcultures have been expressed as compatible to each other. On the other hand, monosporous mycelia obtained from the fruit-bodies on different hosts exhibit complete antagonism between them. Similar interactions have been observed in case of polysporous mycelia and the isolates. The antagonistic behaviour between the mycelia obtained from different host species directly indicate the presence of strain variation within the species. Such intersterility between forms, strains and varieties of the same species has also been reported by Verrall (1934), Arnold (1935), Biggs (1937) and others in different fungi.

Host-relations

It has been found by several workers that aggressiveness and virulence of different strains of a species are not always uniform so far as their host-

relations are concerned. To establish this relationship, experiments have been carried out in order to evaluate the activities of the different strains of *Irpex flavus* on certain selected host species. Cross-inoculation experiments have been made and the method adopted by Banerjee (1955) for the decay-resistance-tests has been followed but with some modifications.

Sound and rectangular wood blocks ($2'' \times \frac{1}{2}'' \times \frac{1}{2}''$) were cut from the sapwood portions of the logs of *Shorea robusta*, *Swietenia mahagoni* and *Albizzia lebbek*. These were examined both macroscopically and microscopically for their soundness and compared with the decayed wood blocks for confirmation. They were serially numbered and dried to constant weights in an oven at 60°C. They were then soaked in distilled water under reduced pressure in order to raise quickly their moisture-content much above the 'fibre-saturation-point'.

The test-blocks were sterilized in an autoclave for 15 minutes at 15 lbs. pressure. About a fortnight before exposing the test-blocks to the strains of the test fungus, several 2% malt-agar slants were prepared in Kolle flasks, each containing about 90 c.cm. of the medium. Different sets of Kolle flasks were inoculated either with the monosporous mycelium or the isolate of the different strains under consideration and incubated at 20°C. In about ten days the mycelia covered the surfaces of all the slants. At this stage, the sterilized sapwood blocks of *Shorea*, *Swietenia* and *Albizzia* were separately introduced into the flasks and placed serially over the growing mycelia. The test-blocks of each host were exposed to six different cultures (monosporous mycelia and isolates of three strains) and incubated for a period of four months at 20°C. and diffused light of a temperature controlled culture room. In order to obtain significant result, this period was allowed following the recommendation of Cartwright and Findlay (1946). Another set of Kolle flasks with test-blocks, but without the test fungus, were kept under identical conditions and these served as controls.

The average moisture-contents of the test-blocks of *Shorea*, *Swietenia* and *Albizzia* before the experiment were determined to be 44%, 54% and 70% respectively. In all cases they were obviously much above the 'fibre-saturation-point' and as such favourable for the fungal activity.

The final average moisture-contents of the test-blocks of *Shorea*, *Swietenia* and *Albizzia* at the end of the experimental period were also determined. It was found that the moisture-contents increased to 65%, 102% and 140% respectively. This may possibly be due to further absorption of water from the medium.

At the end of the test period, the wood blocks were taken out from the Kolle flasks, the superficial mycelia were carefully removed and then dried to constant weights. The difference between the initial and final oven dry weights gave the amount of loss in four months in each case. The results are given in Table 4.

Table 4. Comparison of the average loss in dry weights of sapwood blocks of *Shorea robusta*, *Swietenia mahagoni* and *Albizzia lebbek* due to different monosporous mycelia and isolates of three strains of *Irpex flavus* after 4 months' test.

Types and sources of mycelia	Average loss in % with S.E.		
	<i>Shorea</i>	<i>Swietenia</i>	<i>Albizzia</i>
Monosporous (<i>Shorea</i>)	10.857±0.429	11.965±0.517	19.225±2.024
Monosporous (<i>Swietenia</i>)	4.673±0.672	13.402±0.408	14.248±2.013
Monosporous (<i>Albizzia</i>)	3.600±0.251	5.835±0.235	10.841±0.811
Isolate (<i>Shorea</i>)	6.272±0.358	6.797±0.485	8.313±0.466
Isolate (<i>Swietenia</i>)	5.752±0.466	16.905±0.514	17.801±0.690
Isolate (<i>Albizzia</i>)	4.560±0.365	7.928±0.543	15.128±1.064

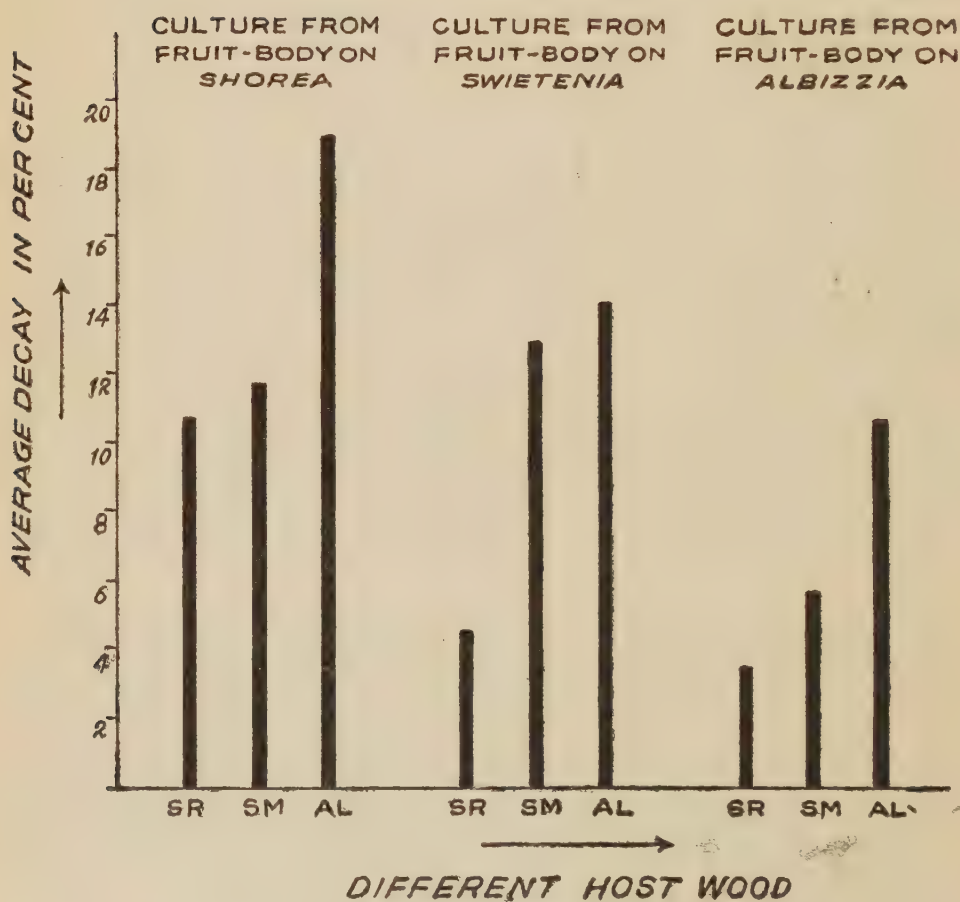
The following conclusions can be drawn from the results embodied in Table 4: (1) there exists a definite relation between the strains under consideration and the aforesaid hosts, (2) different strains with same type of mycelium (monosporous or isolate) differ widely in their aggressiveness and virulence on the same host-species and (3) the type of mycelium, viz., monosporous or isolate is responsible for the variation in their rate of decay on the wood.

It is evident from the results that the activities of all the strains are maximum on *Albizzia*, moderate on *Swietenia* and minimum in case of *Shorea*. Hence, the *Albizzia* may be considered as least resistant and *Shorea* most resistant among the three hosts, while the other may be placed in the intermediate category. This variation in the amount of decay may be due to variation in their physical and chemical properties. Similar variation was also noted by Baxter (1925) while working with *Polyporus hispidus*.

It has also been found that the different strains of the same type of mycelium differ in their decaying activities on the same host-species. The average loss in dry weight by *Shorea* is 10.857±.429, 4.673±.672 and 3.600±.251 per cent by monosporous mycelia obtained from the fruit-bodies growing on *Shorea*, *Swietenia* and *Albizzia* respectively. In case of *Swietenia*, the average loss is 11.965±.517, 13.402±.408 and 5.835±.235 by the monosporous cultures prepared from the fruit-bodies collected from the aforesaid hosts in the same order. *Albizzia*, on the other hand, similarly shows 19.225±2.024, 14.248±2.013 and 10.841±.811 per cent loss in four months. According to Findlay (1938) any timber showing 10–30% loss in four months' test is considered as 'non-resistant' and the average losses in weight up to about 5% is called 'resistant'. So in this case same host may be even 'non-resistant' to one strain and 'resistant' to the other. This variation in the amount of decay is possibly to be due to the different physiological condition of the strains obtained from different host species (TEXT-FIGS. 1 & 2).

GRAPH SHOWING THE RELATIVE VARIATION
IN DECAY OF THE SAPWOOD OF *SHOREA ROBUSTA*
SWIETENIA MAHAGONI & *ALBIZZIA LEBBEK* CAUSED
BY DIFFERENT MONOSPOROUS MYCELIA OF
IRPEX FLAVUS KL.

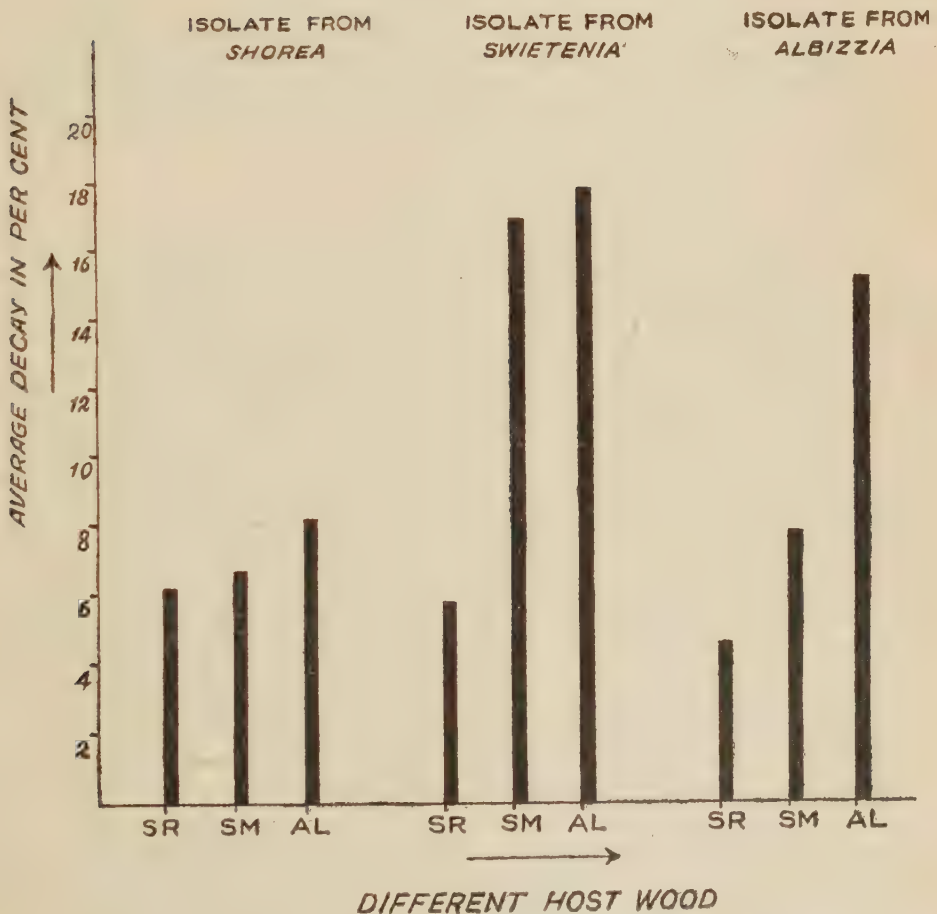
SR = *SHOREA* SAPWOOD
SM = *SWIETENIA* SAPWOOD
AL = *ALBIZZIA* SAPWOOD



TEXT-FIG. 1.

GRAPH SHOWING THE RELATIVE VARIATION
IN DECAY OF THE SAPWOOD OF *SHOREA ROBUSTA*,
SWIETENIA MAHAGONI & *ALBIZZIA LEBBEK* CAUSED
BY DIFFERENT ISOLATES OF *IRPEX FLAVUS* KL.

SR = *SHOREA* SAPWOOD
SM = *SWIETENIA* SAPWOOD
AL = *ALBIZZIA* SAPWOOD



TEXT-FIG. 2.

It is also obvious from the results that the isolates from *Swietenia* and *Albizzia* are rapid decayers than the corresponding monosporous mycelia. But in case of *Shorea*, monosporous mycelium is rapid decayer than its corresponding isolate. The explanation of this variation in the amount of decay caused by the different types of mycelia on the same host species is still awaiting investigation. However, from the above statement it can be said that the natural resistance of timbers not only depends on the kind of wood or types of mycelia or in some cases moisture-content of the wood blocks but also on the nature of the strains of the same type of mycelium of a species.

DISCUSSION

Any investigation on the existence of different strains within a species is an important aspect for evaluating the degree of their decaying activities on different host-species. A study on *Irpex flavus* in relation to various timber-decay makes it possible to discuss some of the points regarding the differential behaviour of its strains and also their relationships with different host-species under consideration.

The fructifications which have been found to grow on logs of *S. robusta*, *S. mahagoni* and *A. lebbek* are more or less identical in their external appearance but when the isolates from the aforesaid hosts are paired, in every case they show antagonism between the interacting mycelia excepting when they are paired with their own sub-cultures. Similar results have also been obtained with the mycelia of monosporous and polysporous origin from different fructifications. No hyphal fusion takes place in any of the pairings. Furthermore, in every case a sub-culture from the line of contact of an interacting pair always yields original cultures by separation. Such intersterility between the mycelia of the same species are, however, not uncommon as have been previously reported by Mounce (1929), Verrall (1934), Arnold (1935), and others.

From cross-inoculation experiments, it has been revealed that all the strains are not exactly uniform in their decaying activities on the same or on different hosts. The sapwood of *S. robusta* shows $10.857 \pm .429\%$ loss in dry weight due to monosporous mycelium of one strain (*Shorea*) but $4.673 \pm .672\%$ and $3.600 \pm .251\%$ losses respectively due to other two strains (*Swietenia* and *Albizzia*). According to Findlay's classification (1938), therefore, *Shorea* is 'non-resistant' to the strain obtained from *Shorea* but 'resistant' to other strains. *Swietenia*, on the other hand, shows $11.965 \pm .517\%$ and $13.402 \pm .408\%$ losses respectively due to two strains (*Shorea* and *Swietenia*) and $5.835 \pm .235\%$ by the other strain (*Albizzia*). But in this case, *Swietenia* is 'non-resistant' to the strains obtained from *Shorea* and *Swietenia* but 'moderately resistant' to the remaining one. *Albizzia* undergoes $19.225 \pm 2.024\%$, $14.248 \pm 2.013\%$ and $10.841 \pm .811\%$ losses by all the three strains (*Shorea*, *Swietenia* and *Albizzia*) respectively and hence this wood is considered to be 'non-resistant' to all of them. Similar differential behaviour is also evident in cases of the isolates obtained from three different hosts. The decaying ability

of the isolates are, however, not similar to that by the monosporous mycelia. It has been found that the isolates from *Albizzia* and *Swietenia* are rapid decayers than that from *Shorea* and their corresponding monosporous mycelia. But the isolates from *Shorea* show less ability to decay than the other two isolates, and its corresponding monosporous mycelium. Thus, on the basis of the average percentage of losses in dry weights it can be said that the sapwood of *Albizzia* is 'least resistant' in comparison with the sapwood of other two hosts as the available strains irrespective of the types of mycelia exhibit maximum decay in this case. Of the three, *Shorea* appears to be the most 'resistant' while the *Swietenia* may be placed in the intermediate category. It is not desirable to generalize from the above statement that any strain of *Irpex flavus* will show the same effect on the aforesaid hosts or the available strains will maintain their same relationship with the other host species. The reason for the differential behaviour of the strains on the same wood may be due to their different physiological conditions. Of course, it is often suggested that the physiological characteristics may possibly be governed by the genetic factors, but this fact still awaits further investigation.

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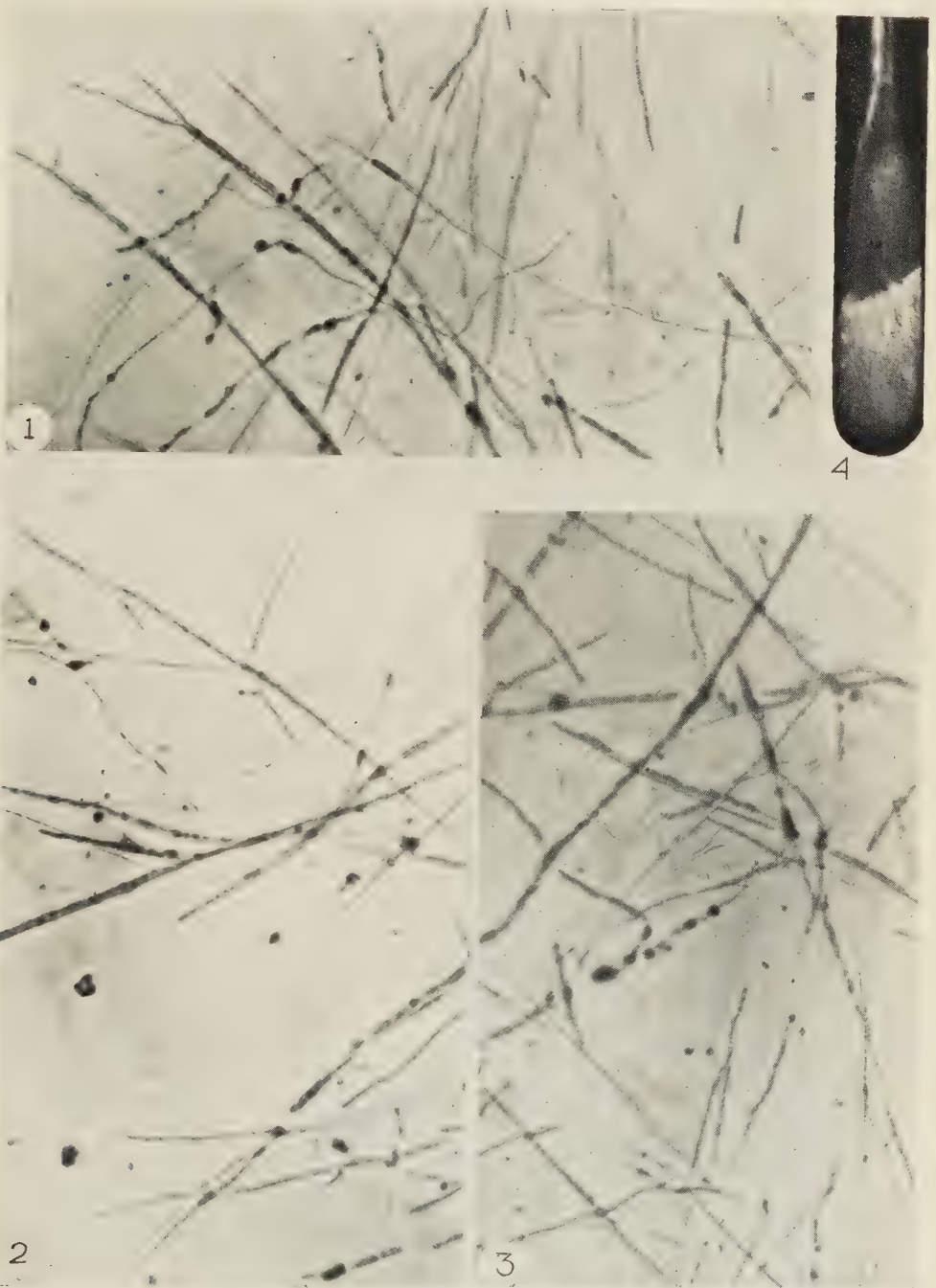
EXPLANATION OF PLATES

PLATE I

- Fig. 1. Photomicrograph of 4-days-old paired cultures of *Irpex flavus* on 2% malt-agar showing overlapping without hyphal fusion between the monosporous mycelia obtained from the fruit-bodies on *Shorea* and *Swietenia*. ($\times 240$).
- Fig. 2. Photomicrograph of 4-days-old paired cultures of *Irpex flavus* on 2% malt-agar showing overlapping without hyphal fusion between the monosporous mycelia obtained from the fruit-bodies on *Albizzia* and *Shorea*. ($\times 240$).
- Fig. 3. Photomicrograph of 4-days-old paired cultures of *Irpex flavus* on 2% malt-agar showing overlapping without hyphal fusion between the monosporous mycelia obtained from the fruit-bodies on *Swietenia* and *Albizzia*. ($\times 240$).
- Fig. 4. Photograph showing separation of original cultures from the inoculum taken from the line of contact of the two monosporous mycelia of *Irpex flavus* obtained from the fruit-bodies on *Albizzia* and *Swietenia*.

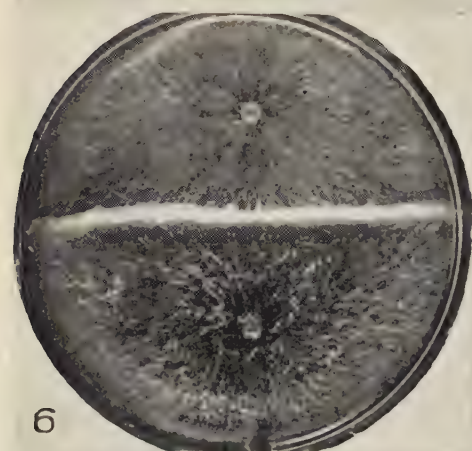
PLATE II

- Fig. 5. Photograph of 7-days-old paired cultures of *Irpex flavus* on 2% malt-agar showing the white line of demarcation between the isolates from *Swietenia* and *Shorea*.
- Fig. 6. Photograph of 7-days-old paired cultures of *Irpex flavus* on 2% malt-agar showing white line of demarcation between the isolates from *Albizzia* and *Shorea*.
- Fig. 7. Photograph of 7-days-old paired cultures of *Irpex flavus* on 2% malt-agar showing the white line of demarcation between the isolates from *Albizzia* and *Swietenia*.
- Fig. 8. Photograph showing 14-days-old paired cultures of *Irpex flavus* on 2% malt-agar showing the dark coloured area between the monosporous mycelia obtained from the fruit-bodies on *Shorea* and *Swietenia*.
- Fig. 9. Photograph showing 14-days-old paired cultures of *Irpex flavus* on 2% malt-agar showing the dark coloured area between the monosporous mycelia obtained from the fruit-bodies on *Albizzia* and *Shorea*.
- Fig. 10. Photograph showing 14-days-old paired cultures of *Irpex flavus* on 2% malt-agar showing the dark coloured area between the monosporous mycelia obtained from the fruit-bodies on *Albizzia* and *Swietenia*.



Irpex flavus

S. N. Banerjee & R. P. Purkayastha



CONIOPHORA CEREBELLA PERS., A DRY ROT FUNGUS ON BUILDING TIMBER IN INDIA

By

B. K. BAKSHI, BALWANT SINGH AND P. S. REHILL

Forest Research Institute, Dehra Dun

(With PLAE I and 1 TEXT-FIGURE)

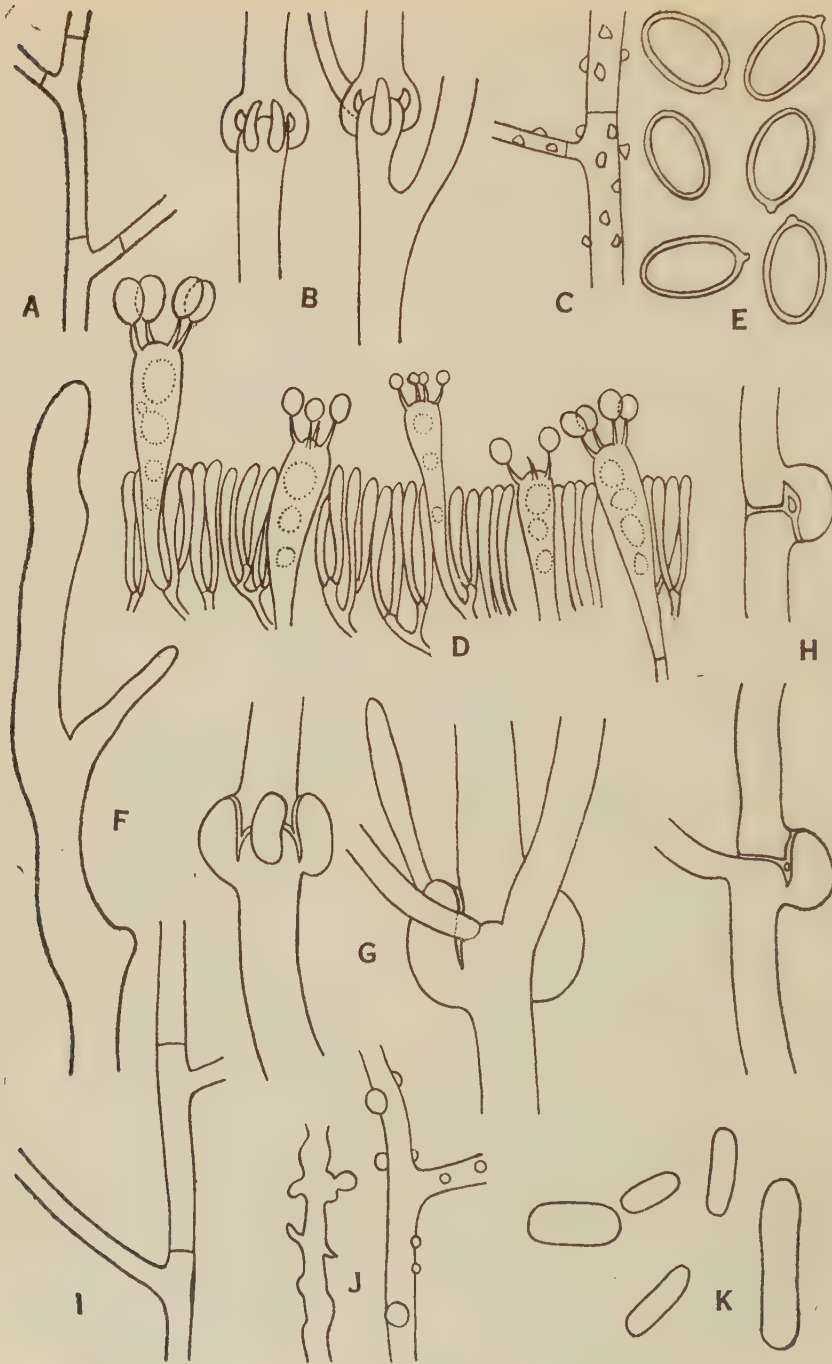
Coniophora cerebella is common in Europe and also occurs in America and Australia. The fungus causes decay in logs and building timber under very moist conditions and the decay is referred to as 'wet rot'. It is usually used as a test-fungus in laboratory tests on the durability of treated and untreated wood. The present collection of the fungus was made from a Forest Rest House at Konain (Alt. 6,500 ft.), Chakrata Forest Division, Uttar Pradesh, during October, 1957. The sporophore (PL. I, FIG. 1) was growing on wooden floors of kail (*Pinus wallichiana*) in contact with the ground and also on ceilings. In the present paper the Indian collection of the fungus, the first of its kind, has been described from sporophore and culture.

Sporophore

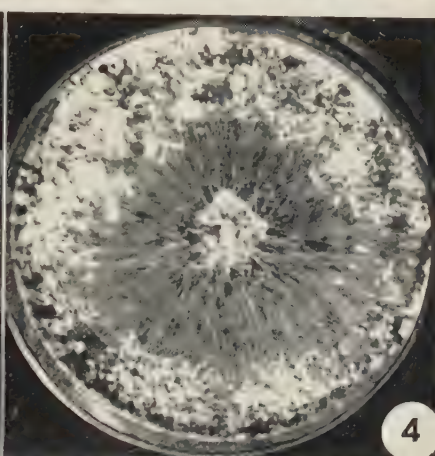
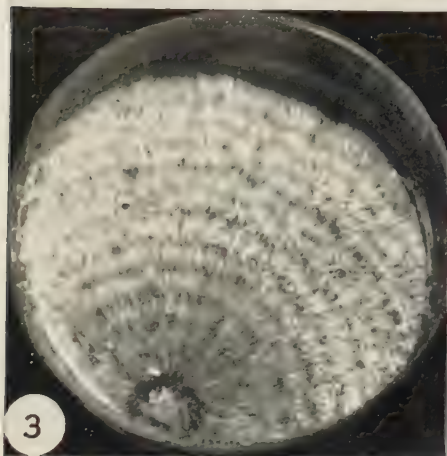
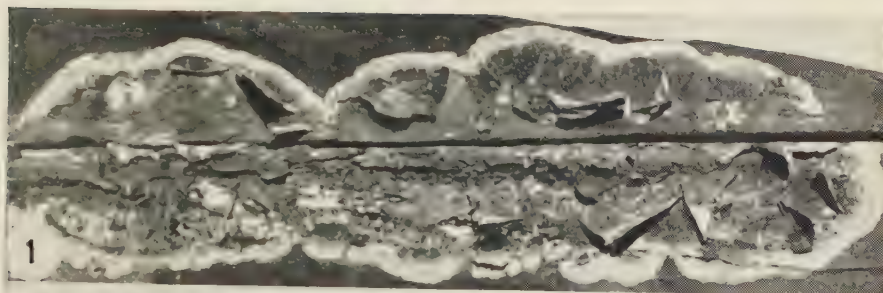
Sporophore membranous, pulverulent, usually broadly effused, mature areas separating from the substratum. *Hymenial surface* cream yellow when young turning olive brown with the development of spores, tuberculate in fresh specimens, even and cracking when dry. *Margin* white, thin. *Subiculum* upto 1.4 mm. thick, composed of densely interwoven hyphae without any differentiation into layers; *hyphae* of two types (a) hyaline, branched, septate, thin or slightly thick-walled, without clamp-connexions (TEXT-FIG. 1,A), $3.5-5\mu$ wide, present mostly in the subhymenial region, and (b) hyaline, branched septate, thin or slightly thick-walled, with whorled clamp-connexions, 3-6 in each whorl (TEXT-FIG. 1,B), $7.8-10.5\mu$ wide, present usually in the growing margin of the fruit-body and also in the subiculum towards substratum, often incrustated with crystals in the growing region and in the region adjoining the substratum (TEXT-FIG. 1,C). *Hymenium* $40-60\mu$ thick, composed of basidia and paraphyses (TEXT-FIG. 1,D). *Basidia* clavate to sub-clavate, upto 50μ long and $7-11\mu$ wide, 4-spored sterigmata upto 8μ long. *Basidiospores* smooth, elliptical or obovate, apiculate (TEXT-FIG. 1,E), dark brown in mass, pale brown individually, $10-14.9 \times 5.25-7.8\mu$.

Rot

The fungus causes brown cuboidal rot (PL. 1, FIG. 2). Dark brown slender strands are present on the surface of the decayed wood and also in the crevices of the rot.



TEX-FIG. 1. Microscopic characters of *C. cerebella* : A-C & E-K ($\times 1250$), D ($\times 540$).
 (For explanation vide text)



Coniophora cerebella

Bakshi, Singh & Rehill

Fungus in culture

GROWTH CHARACTERS. Growth rapid, in one week radial growth on *malt-agar* in darkness 3.5–4.2 cm. at 26°C., 4.0–4.5 cm. at 24°C., 2.8–3.4 cm. at 18°C. and nil at 5°C. (but 0.5 cm. in 4 weeks). Advancing zone hyaline, even. Mat white, or cartridge buff, maize yellow and cream buff, colour more prominent in young culture, tints of ‘tawny olive’ developing at places when old, cottony, raised, forming concentric zones, zones more prominent when inoculum placed on one side of Petri dish (PL. I, FIG. 3) than that when placed at its centre (PL. I, FIG. 4), frequently forming condensed patches along the sides of Petri dish, mature region becoming adpressed and showing radiating strands; reverse changing to chamois or honey yellow and later ‘cinnamon brown’. Odour strong, unpleasant. On gallic and tannic acid agars no diffusion zones, radial growth 3.5–4.0 cm. in the former and 1.5–2.0 cm. in the latter. On *gentian-violet-agar* growth fast and medium not discoloured.

HYPHAL CHARACTERS. *Advancing zone.*—Hyphae hyaline, thin to slightly thick-walled (TEXT-FIG. 1,F), usually with multiple clamp-connexions forming whorl of branches at the nodes (TEXT-FIG. 1,G), 5–12 μ wide, rarely with single clamp-connexion (TEXT-FIG. 1,H), frequently branched with simple septa, branches 1.5–4.5 μ wide. *Aerial mycelium.*—Hyphae broad, with whorled clamp-connexions and narrow branches with simple septa (TEXT-FIG. 1,I) as in the advancing zone. Brown hyphae, slightly thick-walled with small protuberances (TEXT-FIG. 1,J), 2–4.5 μ broad also present; oidia (TEXT-FIG. 1,K) of variable width (2–5 μ). *Submerged mycelium.*—Hyphae hyaline, branched with simple septa as in aerial mycelium.

The identification of the present collection as *C. cerebella* was confirmed by Dr. Mildred Nobles, Ottawa, Canada, from culture and by Dr. L. K. Weresub from sporophore. Our sincere thanks are due to them.

(Received for publication 1 September 1958)

EXPLANATION OF PLATE

- FIG. 1. Sporophore of *Coniophora cerebella* ($\times 0.36$).
FIG. 2. Brown cuboidal rot with strands (s) ($\times 1$).
FIG. 3. 2-weeks-old culture showing concentric zones, ($\times 0.6$).
FIG. 4. 2-weeks-old culture with zones not evident ($\times 0.6$).

SPORULATION AND THE MECHANISM OF SPORE-DISCHARGE IN *TRAMETES LACTINEA* BERK.

By SACHINDRANATH BANERJEE

AND

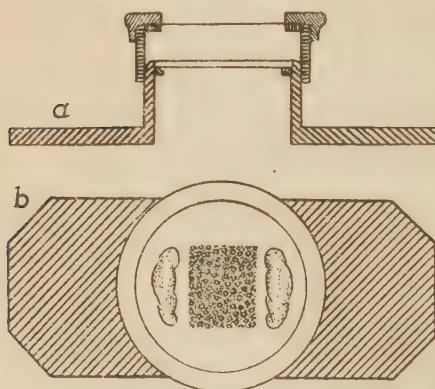
NARAYAN CHANDRA BANERJEE

Department of Botany, University of Calcutta

The production and liberation of spores are of fundamental importance to a fungus and essential to the perpetuation of species. Different groups of fungi adopt diverse means by which the spores are formed and expelled from the fruiting bodies for dissemination. The members of Hymenomycetes, especially the Polyporaceae, afford many interesting problems so far as the functions of these special adaptations are concerned. The minute size of the basidium and the basidiospores together with their extreme delicacy and peculiar arrangement in the hymenium make it difficult to study this aspect of the life-cycle. Buller (1909), however, carried out researches on the mode of sporulation and mechanism of spore-discharge in a number of Hymenomycetes. The present investigation on *Trametes lactinea* Berk., adds further details to our present-day knowledge of the phenomenon of spore-discharge of Polyporaceae.

For this study the writers have used a special apparatus known as the "Compressor Cell" as adopted by Buller (1909). The apparatus consists of a flat rectangular metallic stage with a central hole and a thick, circular glass plate mounted on a ring occupying the rim of the hole. The glass plate forms the base of a chamber which is covered by a lightly fitting cap over it. The cap bears a circular cover-glass at the centre (TEXT-FIG. 1).

Fresh and well-developed fruit-bodies were collected from the field and moistened by placing on their upper surface some absorbent cotton



TEXT-FIG. 1. Diagrammatic representation of the sectional (a) and top (b) views of a "Compressor Cell."

soaked with distilled water in the laboratory for about half an hour to obtain the optimum moisture-content necessary for sporulation. For each observation a thin transverse section through the hymenium was cut with a dry razor and placed on the stage of the 'compressor cell'. Two moist wads of cotton were placed on two sides but not in contact with the section. The stage was then covered with the cap and adjusted in such a way that the cap just touched the section without any pressure in order to keep it flat over the surface of the stage. In the

closed and moist cavity thus produced, the section showed basidia projecting horizontally inwards from the sides of the pore-tubes. Extreme care was taken in cutting sections and in their further handling in order to avoid distortion of hyphae and basidia. Constant observation was then carried out under a compound microscope.

The hymenium consists entirely of basidia. All the basidia are not of the same age and they are of different developmental stages. They grow in gradual succession and thus continue sporulation for a long time. It may be mentioned that sections of the fruit-body which have already started sporulation are best suited for studying the developmental stages of the basidia. The mature basidia remain somewhat raised from the general level of the hymenium and appear to be more turgid and stouter than the younger ones. They are tetrasterigmatic and quadrisporous. The sterigmata are cruciately arranged on the top of the basidium and are long and pointed at their apices.

The early stage of the spore-formation is revealed by the appearance of minute rounded saes right over the apices of the sterigmata. They are hyaline and enlarge very slowly taking about four to five hours to attain the full size of the spores. Though they start their development simultaneously, all of them do not attain maturity at the same time. Each mature basidiospore, thin-walled and elliptic, remains attached to the apex of the sterigma near the pointed hilum. After attaining full size the spores are not immediately discharged but remain attached to the sterigmata for a period of one to two hours. A very minute water-drop then suddenly appears at the hilum of each spore. The spore is then shot out violently from the sterigma with an initial horizontal force. With this initial velocity the spore travels forward horizontally to a distance of about 46–67 μ , about 9–14 times the length of the spore itself. Just before spore-discharge the tips of the sterigmata deflect slightly in diagonally opposite directions. Extreme difficulties were encountered in tracing the travelling path of the individual spore after its discharge. This is due to the fact that it does not come in the same focus. However, by adjusting the microscope up and down, the spores attached to the stage or the cover-glass of the apparatus can be found and the travelling distance was measured by an ocular micrometer. The horizontal distance which the spores travel can be correlated with the diameter of the pore-tube and has been found to be approximately the half of its diameter. As soon as their horizontal velocity ceases to exist, the spores fall downwards passively through the space of the pore-tube.

All the four spores of a basidium are not simultaneously discharged, instead they are expelled successively one after another and the whole operation is completed within an hour or so. The time interval between the two successive discharges of spores is about 10 to 20 minutes. Consequently, mature basidia with one, two, three or all the four spores attached can be found in the same hymenium in a single focus. After discharging the spores the empty basidia gradually lose their turgidity, become somewhat collapsed and are ultimately drawn down to the general level of the hymenium. It has also been observed that all the four spores of a basidium

are not always discharged. Sometimes basidium with one or two spores still attached has been found to collapse. In some cases basidium does not discharge its spores at all. In no case, however, basidia producing two generations of spores are noticeable.

From the above observation it is evident that the spore-discharge in *Trametes lactinea* is well oriented with the shape and direction of the pore-tubes. Again, it is co-ordinated with the space within them. The spores are violently projected towards the centre of the pore-tubes and then fall down due to gravity. As a result, they never get attached to any surface within the pore-tubes and are ejected outside. The successive ejection of spores in somewhat different directions is of great advantage since this avoids their coherence among themselves. A further illustration of the above fact can be shown by allowing a piece of sporophore to discharge its spores on a black paper under a bell jar. A beautiful spore print coinciding with the pore-tubes is obtained.

The casting of spores in a well-developed fruit-body is a continuous process. Sporulation in the field, however, has been observed to be more heavy at certain periods than at others. If the fruit bodies have their optimum moisture-content for sporulation and if it is a bright sunny day with occasional showers during the early part of the day, the frequency of sporulation is maximum at noon. Clouds of spores are emitted and the air beneath the sporophore being full of spores gives a smoky appearance even to naked eyes. This liberation of spores in smokes continues unaffected till evening provided the external conditions are not unfavourable. Torrential rain with stormy wind, sudden fall or rise of temperature and certain other biotic factors have inhibiting effects upon the phenomenon. The spore-clouds, however, are carried to a little distance away from the fruit-bodies and disseminated by the wind under normal conditions. This remarkable liberation of spores from the fruit-bodies of polypores has also been reported by Buller (1909) particularly in *Polyporus squamosus* and by White (1919) in *Fomes applanatus*. It has not been possible to observe the phenomenon at night in the field, but fruit-bodies liberated spores in great profusion during night, specially at midnight, under ordinary environmental conditions of the laboratory. Another interesting fact should be mentioned that so far as the production of spores is concerned, the ability of the different parts of the sporophore varies greatly. In comparing the sporulating activities from margin towards the base, it has been found that it is the middle region of the fruit-body which sporulates most vigorously and continues to do so for longer period of time.

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A PRELIMINARY NOTE ON VITAMIN DEFICIENCY IN *POLYSTICTUS SANGUINEUS* (L.) MEY.

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It is now a well established fact that most of the fungi require for their growth one or more vitamins (Lilly and Barnett, 1951). Some fungi have the ability to synthesize certain vitamins but others must depend upon a supply from the substrate on which they grow. The response of *Polystictus sanguineus* (L.) Mey., growing in pure culture, to an exogenous supply of four vitamins is reported in this paper.

This investigation was made with a culture obtained from a freshly collected specimen by tissue-culture method. A glucose-asparagine medium as described by Sinha (1956) and previously found to be a good growth-supporting medium for this fungus was used throughout. In its preparation the basal medium, minus micro essential elements, was boiled with activated charcoal (5g. per litre) to remove any vitamin present. Micro essential elements and vitamins were added after this treatment. Vitamins used in this investigation were added to the medium in the following concentrations: thiamine, 100 μ g./litre; biotin, 5 μ g./litre; pyridoxine, 100 μ g./litre and nicotinamide, 100 μ g./litre. The fungus was tested in a series of six different treatments. Four media were prepared and in each of them one vitamin was absent. In addition, 2 control media were employed, one consisting of basal medium with all 4 vitamins and the other without any vitamin. The pH of the medium was adjusted to 6.0 in each case. 20 ml. portions of media were distributed into Erlenmeyer flasks of 150 ml. capacity. Sterilization of the medium was done by autoclaving for 15 minutes at 15 lb. pressure. Replicates of 6 flasks were employed for each treatment.

Thin and uniform discs (5 mm. in diameter) cut out from the advancing region of actively growing colonies of the test fungus on potato-dextrose-agar medium were used as inocula. The cultures were incubated at $25\pm 0.5^{\circ}\text{C}$. in total darkness for a period of 14 days and kept without shaking. After harvesting the mycelium was dried to a constant weight at 90°C . The results reported as milligrams of dry mycelia, being the averages of 6 cultures, are recorded in the following table.

Table 1. *Growth of Polystictus sanguineus in 20 ml. of liquid glucose-asparagine medium supplemented with various vitamins. Incubation period—14 days.*

Vitamins added	Dry weight in milligrams
All vitamins	47.4
All minus thiamine	10.83
All minus biotin	54.2
All minus pyridoxine	58.8
All minus nicotinamide	54.6
None	3.9

It becomes evident from the data that *Polystictus sanguineus* has a requirement for thiamine only. In the absence of thiamine complete inhibition of growth does not take place but the organism grows very slowly. *Polystictus sanguineus*, therefore, appears to have partial deficiency for thiamine. In this case partial deficiency is nearly total and a very slow rate of thiamine synthesis is indicated. Little difference in growth on different media containing thiamine indicates that partial deficiencies for biotin, pyridoxine and nicotinamide do not exist for this fungus. In the control series with vitaminless medium a slight growth was recorded. This naturally leads to a suggestion that thiamine synthesis by *Polystictus sanguineus* may be dependent upon the presence in the medium of one or more of the 3 other vitamins used in this investigation.

The author is deeply indebted to Dr. S. N. Banerjee of the College of Science, Calcutta University, for supplying the material for study. His grateful thanks are also due to Dr. S. N. Bhaduri and Sri D. R. Chatterjee of the Government College, Darjeeling, where this work was carried out, for their help.

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EFFECT OF GROWTH-SUBSTANCES ON *MERULIUS SIMILIS* B. & BR. IN CULTURE

By

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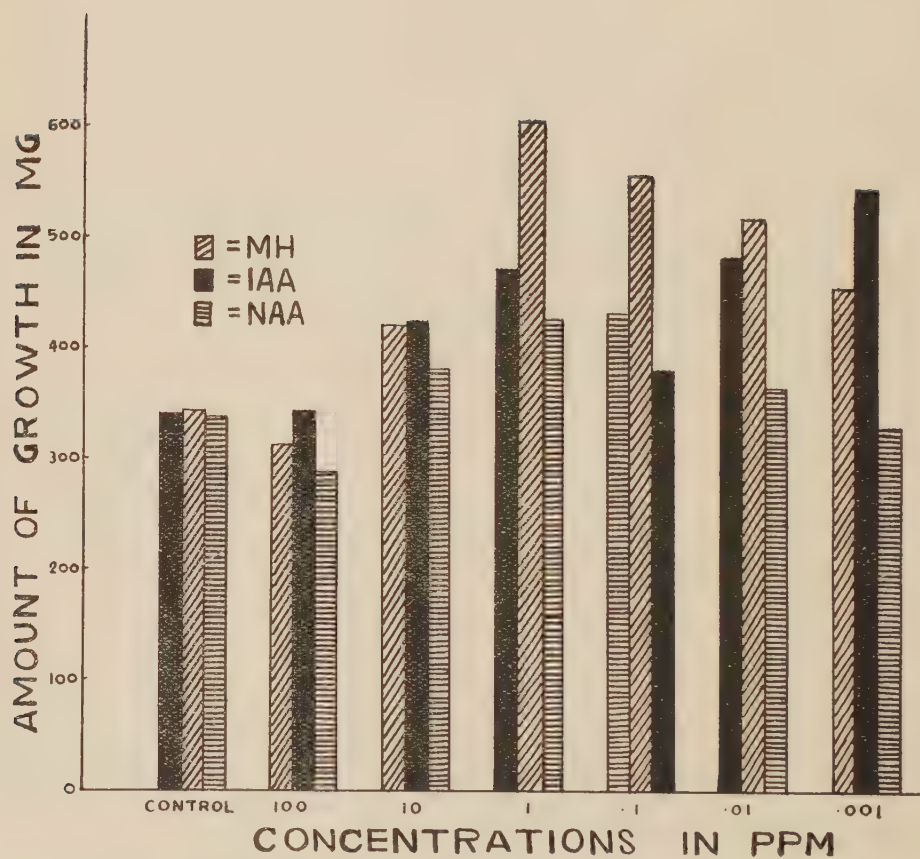
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The application of growth-substances to plants has received much emphasis in recent years. The multifarious effects of auxins and anti-auxins on the growth of the root and stem, production of inflorescence, formation of anther and pollen-grains and setting of fruits and seeds have been extensively studied. But little attention has been paid so far to the effect of these substances on the growth of the fungal mycelium.

It is generally agreed that growth-substances are generally required in minute doses, but in higher concentration they usually inhibit growth. Leonian and Lilly (1937) have come to the same conclusion after testing about one hundred fungi with regard to the effect of β -indole acetic acid. Merhotra (1951) has also found this inhibiting effect without a single exception in the concentration higher than one part per 10 million of the growth-promoting substances tried on species of *Phytophthora*.

In order to study the effects of different growth substances on *M. similis*, two commonly used auxins, indole-acetic acid and α -naphthalene-acetic acid and an anti-auxin, maleic hydrazide, were taken. A graded concentrations of 0.001 PPM, 0.01 PPM., 0.1 PPM., 1 PPM., 10 PPM. and 100 PPM., were prepared with each of them employing *potato-dextrose* as the basic liquid medium. This medium might contain an appreciable amount of these substances, but as all the grades were prepared with the same medium, the results might, therefore, be comparable.

Fresh sporophores of *M. similis* were collected during the months of the rainy seasons and stock cultures were made from them in 2% malt-agar slants. The grades of the growth-substances after preparation were poured in separate Erlenmeyer's flasks (250 ml.), each containing 100 ml. of each grade. They were plugged and sterilized at 15 lbs. pressure for 10 minutes. The flasks after sterilization were inoculated with uniform agar-discs obtained from the culture and were incubated at 25°C., for a period of seven days in diffused light of the laboratory. Three replicates for each grade were prepared with adequate number of controls. After the period of incubation, the mats were taken out, washed thoroughly and dried at 60°C. to constant weights. The results thus obtained are given in the Tables 1-6 and TEXT-FIG. 1,



TEXT-FIG. 1. Effect different concentrations growth substances on the amount of growth of *M. similis*

Table 1. *Effect of different concentrations of IAA on the amount of growth.*

Replication Grade (PPM)	Dry weight (in mg.)			
	I	II	III	Average
0.001	540.000	545.250	538.680	541.976
0.01	482.015	480.950	485.100	482.688
0.1	432.070	436.580	428.900	432.496
1	430.005	433.020	424.920	424.248
10	425.000	418.540	420.950	421.496
100	340.032	338.005	345.830	341.289
Control	340.000	340.000	340.050	340.016

Table 2. *Analysis of variance on the results obtained due to variation in concentration of IAA.*

Source of variation	D.F.	S.S.	Mean S.S.	'F' Value
Between treatment	6	107434.74	17905.79	38.69**
Residual	14	6479.02	462.78	
Total	20	113713.76		

The 'F' value is highly significant at both 5% and 1% level of critical difference.

Table 3. *Effect of different concentrations of NAA on the amount of growth*

Replication Grade (PPM)	Dry weight (in mg.)			
	I	II	III	Average
0.001	320.000	324.340	322.950	322.430
0.01	362.010	360.580	566.120	362.903
0.1	380.000	378.898	380.005	379.634
1	470.010	470.000	470.000	470.003
10	382.000	385.005	381.001	382.668
100	290.000	285.500	288.250	287.916
Control	340.000	338.920	340.000	339.973

Table 4. *Analysis of variance on the results obtained due to variation in concentration of NAA.*

Source of variation	D.F.	S.S.	Mean S.S.	'F' Value
Between treatment	6	59641.47	9940.24	516.37**
Residual	14	26.95	1.92	
Total	20	59668.42		

The variation due to different concentration is highly significant at both 5% and 1% level of critical difference.

Table 5. *Effect of different concentrations of MH on the amount of growth.*

Replication	Dry weight (in mg.)			
	I	II	III	Average
Grade (PPM)				
0.001	460.007	455.680	450.650	455.445
0.01	510.017	515.980	518.320	514.772
0.1	552.010	550.750	554.001	552.253
1	600.000	596.340	605.005	600.448
10	420.025	416.725	425.640	420.796
100	312.032	314.000	314.000	313.344
Control	340.000	341.910	342.500	341.470

Table 6. *Analysis of variance on the results obtained due to variation in concentration of MH.*

Source of variation	D.F.	S.S.	Mean S.S.	'F' value
Between treatment	6	204847.05	34141.17	2746.6**
Residual	14	174.05	12.43	
Total	20	205021.10		

The variation due to different concentration is highly significant at both 5% and 1% level of critical difference.

From Table 1, it is evident that the amount of growth in IAA is maximum in the grade 0.001 PPM. and it gradually decreases with further increase in concentration. But in all cases except in 100 PPM, the amount is more than that in the controls, while in 100 PPM it is more or less the same as the control value. From this it can be said that optimum concentration of IAA can induce better growth, but no pronounced inhibition of growth takes place at higher concentrations up to 100 PPM.

From Table 3, it is event that the amount of growth increases with increase in concentration up to 1 PPM above which it sharply decreases and at 100 PPM pronounced inhibition of growth has been found in case of NAA.

From Table 5, it has been found that in MH the amount of growth increases with increased concentrations and best growth has been obtained at 1 PPM. With further increase in concentration, the amount decreases and at 100 PPM pronounced inhibition is observed.

In all these cases, the differences in the amount of growth obtained due to variation in concentrations has been found to be highly significant (Tables 2, 4 & 6).

From the comparative study of the Tables, it can be easily visualized that the fungus exhibits maximum amount of growth in MH, where at the optimum concentration the amount of growth is 600.448 mg. MH distinctly inhibits all forms of growth, but in case of this fungus it is curious enough to note 80.21% increase in comparison with the average control value, at the optimum concentration of it. In cases of NAA and IAA, the maximum total amount of growth are 470.00 mg. and 429.315 mg. respectively. The inhibition effect of higher concentrations is more pronounced in case of MH and NAA, but no inhibition of growth has been obtained in the case of IAA.

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FURTHER STUDIES ON USTILAGINALES :

MORPHOLOGY AND SPORE-GERMINATION OF *PERICLADIUM GREWIAE*

By N. C. JOSHI

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Pericladium is an important genus of the family Ustilaginaceae. The genus has been described by Saccardo (1888). Mundkur and Thirumalachar (1952) recorded two species from India. In Ajmer a few species of *Grewia* are attacked by this genus which produces considerable loss to the plants. For two years the writer has studied the symptoms of the disease and spore-germination in detail.

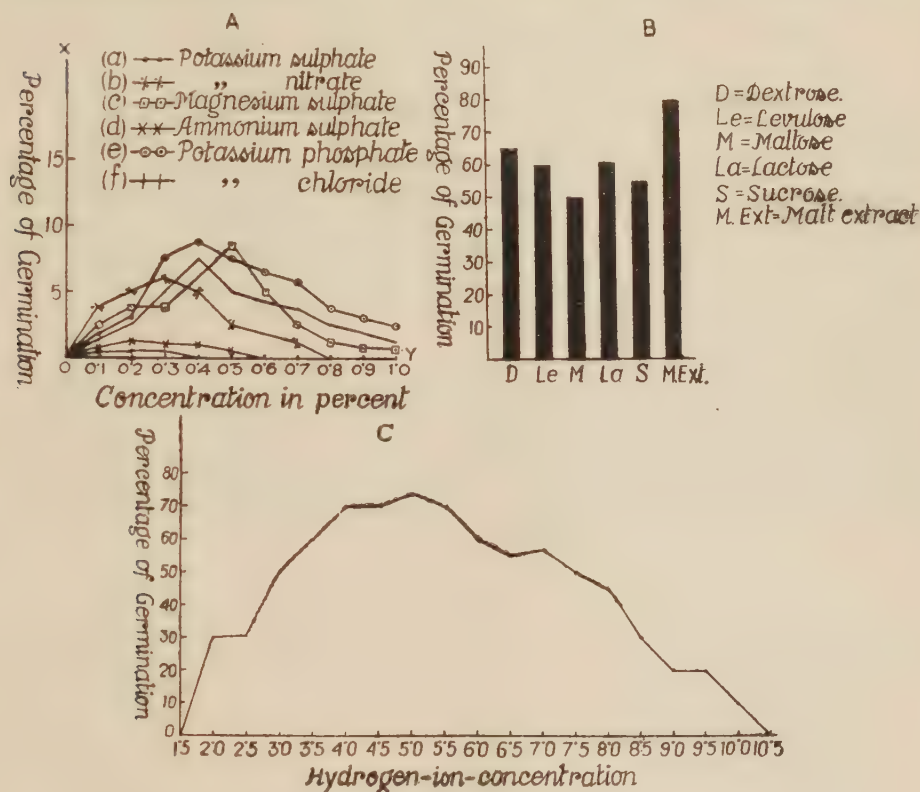
For the present investigation *Pericladium grewiae* was collected from many localities of Ajmer on different species of *Grewia*. The twigs and branches were first dried for 24 hours and then kept in cellophane bags which were later used for spore-germination studies. The salts and sugars used for this investigation were in chemically pure forms.

The symptoms of the disease mainly appear on the petiole, twigs and branches of the plant. Both young and mature plants are susceptible to the disease. The disease can be easily detected by the presence of blister-like pustules, each of which is covered by a coriaceous, hard and woody indusium formed by the host tissue. These blisters increase in size and form bigger pustules. Sometimes the upper branches are severely affected by the disease and appear like the Witch's broom. The diseased plants get deformed and stunted. At maturity the host tissue which covers the pustules degenerates and exposes the powdery mass of black spores. In Ajmer this is a very common sight during the winter months and it persists for a long time. The spore mass is black. Each spore is globose, subglobose, sometimes ellipsoidal, greyish in colour, 5.5 to 9 μ in diameter and with thick and smooth episore.

The spores of this smut readily germinate in the water and in solutions of salts or sugars, but a number of salts proved to be toxic. In ordinary water the spore germinates within 24 hours, its exosporium bursts and endosporium gives rise to a septate promycelium with a single terminal sporidium which easily get detached at a later stage.

Sodium chloride and Sodium carbonate were proved to be toxic and there was no germination even in 0.1% solution of the salts like that in *Sphacelotheca mc Alpineae* Zundel (Joshi, 1957). Ammonium sulphate and Potassium chloride also proved to be highly toxic. The other salts like Potassium sulphate, Magnesium sulphate, Potassium nitrate and Potassium phosphate were not so toxic although the percentage of germination was fairly low. The results are shown in TEXT-FIG. 1-A. The spores germinated in 4% solutions of dextrose, laevulose, maltose, lactose, sucrose

and malt-extract. The maximum germination was observed in 4% malt-extract (TEXT-FIG. 1-B).



TEXT-FIG. 1.—Graph showing rates of spore-germination

Further observations were made on the effect of pH where the spores germinated in 4% lactose solution at room temperature (22° – $23^{\circ}C.$) and having a pH range of 1.5 to 10.5 which was determined by Beckman's pH meter. The results are given in TEXT-FIG. 1-C, and show that the spores were capable of germination in a wide range of pH , i.e., 2.0 to 10.0. The optimum pH was 6.5.

I am grateful to Shri Bhim Sen and Prof. B. Tiagi for the facilities. My thanks are also due to Dr. N. C. Sogani for preparing sugar solutions of different pH with Beckman's pH meter.

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ON THE PERFECT STAGE OF *CHAETOMELLA* EKL.

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It has been shown (Ghosh, 1957) that *Rhizoctonia bataticola* (Taub.) Butler causes root-rot in Jute and the wilting is rapid in jute-potato-fields where a number of other fungi and some bacteria are also associated with it. Isolations from roots of completely wilted plants sometimes yield two types of cultures having identical growth characteristics but one produces pycnidia and the other, perithecia. The descriptions of these fruiting bodies are as follows :—

Pycnidia separate or cespitose, superficial, globose, wall membranous, beset all over with setose hairs, chocolate brown in colour, $116-172 \times 106-154\mu$ (TEXT-FIG. 1, A). *Pycnosporos* numerous, mostly lenticular, somewhat thick-walled, mature spores at first brown but with age steel gray to violet in colour, immature ones hyaline, $10-12 \times 5-6.5\mu$ (TEXT-FIG. 1, B).



TEXT FIG. 1. A, Pycnidium of *Chaetomella* sp.; B, pycnosporos of *Chaetomella* sp. ($\times 320$); C, perithecium of *Chaetomium* sp.; D-E, mature and immature asci of *Chaetomium* sp. ($\times 320$); F, ascospores of *Chaetomium* sp. ($\times 320$); G, Setose hairs ($\times 305$).

Setose hairs many, generally straight and ending in a blunt point, thick-walled, septate, septa usually three in each and with a bulbous base $90-151 \times 5-6.5\mu$ (TEXT-FIG. 1, C).

Perithecia separate to cespitose, superficial, globose to somewhat cylindrical, without any beak, wall membranous and studded with setose hairs, chocolate brown in colour, $106-212 \times 83-181.5\mu$ (TEXT-FIG. 1, C). *Asci* typically clavate to cylindrical, evanescent, 8 spored, $19.5-27.5 \times 6.5-10\mu$ (TEXT-FIG. 1, D-E). *Ascospores* mostly lenticular, one-celled, thick-walled, hyaline when young, becoming oblique-brown on maturation, $10-11 \times 5.5-6.5\mu$ (TEXT-FIG. 1, F). *Setose hairs* many, straight, usually ending in a blunt point, brown, thick-walled, septate, septa usually three in each and with a bulbous base, $30.5-189 \times 2-3.5\mu$ (TEXT-FIG. 1, G).

From the aforesaid descriptions it can be stated that the perithecia bearing culture is a species of *Chaetomium* while that producing pycnidia is a species of *Chaetomella*. Polysporus cultures from ascospores on *potato-dextrose-agar* produced both perithecia and pycnidia in the same tube, but similar transfers of pycnospores did not yield any mixed culture.

From these descriptions it may be stated that from the similarities of morphological characters of the fruit-bodies of both types of isolate, their habitat and constant association in nature, *Chaetomium* may be the perfect state of *Chaetomella*. Further investigations, however, are in progress to confirm this statement.

Thanks are due to Dr. B. C. Kundu, Director, Jute Agricultural Research Institute for giving proper facilities to work and to the Mycologist Sri T. Ghosh for suggestions.

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